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Recent Research on EMF and Health Risk
- Fourteenth report from SSM's Scientific
Council on Electromagnetic Fields, 2019

SSM perspective

Background

The Swedish Radiation Safety Authority's (SSM) Scientific Council on Electromagnetic Fields monitors current research on potential health risks in relation to exposure to electromagnetic fields and provides the Authority with advice on assessing possible health risks. The Council gives guidance when the Authority must give an opinion on policy matters when scientific testing is necessary. The Council is required to submit a written report each year on the current research and knowledge situation.

This is a consensus report. This means that all members of the Scientific Council agree with the complete report. This increases the strength of the given conclusions.

Objective

The report has the primary objective of covering the previous year's research in the area of electromagnetic fields (EMF) and health but also to put this in the context of present knowledge. The report gives the Swedish Radiation Safety Authority an overview and provides an important basis for risk assessment.

Results

The present report is number fourteenth in a series and covers studies published from April 2018 up to and including December 2018 (previous report covered studies published up to and including March 2018). The report covers different areas of EMF (static, low frequency, intermediate, and radio frequency fields) and different types of studies such as biological, human and epidemiological studies.

No new established causal relationships between EMF exposure and health risks have been identified.

Overall, the age standardised incidence of brain tumours does not give support to any causal relationship with radio wave exposure from mobile phone use. If there is an impact, it appears to be so weak that it cannot be detected in incidence trend studies.

Studies on impact on cognitive functions and behaviour of children and adolescents often report associations with the use of wireless information technology. Since the strongest associations have been found correlated to applications which give low exposure to the head, e.g. texting, it seems clear that other reasons than radio wave exposure primarily causes the association. A few attempts have been done in order to discriminate radio wave exposure from other possible sources of impact. In such a study some indications of weak impact of radio wave exposure was found but this observation needs to be confirmed in similar study approaches before any robust conclusions can be drawn.

In line with previous reports the council report studies on increased oxidative stress due to weak radio wave exposure in animal studies, some

even below the reference levels. Increased oxidative stress was observed in the eye, testes and sciatic nerve. However, several studies did not observe oxidative stress in the brain. Oxidative stress is a natural biological process that can sometimes be involved in pathogenesis, but under what circumstances oxidative stress due to weak radio wave exposure may affect human health remains to be investigated.

Some animal studies observed that radio wave exposure of testes resulted in decreased sperm counts, sperm viability and serum testosterone. To what extent such exposure can also affect humans need to be investigated.

A meta-analysis study concluded a slight increased risk for ALS among workers with increased exposure for low frequency magnetic fields.

Despite the increasing use of applications in the intermediate frequency (IF) range of the electromagnetic spectrum (300 Hz-10 MHz) scientific evaluation of potential health risks in that range is scarce.

The annual report also includes a section where studies that lack satisfactory quality have been listed. This year, as well as last year, many studies have been excluded due to poor quality. From a scientific perspective, studies of poor quality are irrelevant. They are also a waste of money, human resources and, in many cases, experimental animals.

Relevance

The results of the research review give no reason to change any reference levels or recommendations in the field. However, the observations of biological effects in animals due to weak radio wave exposure clearly show the importance of maintaining the Swedish Environmental Code¹ precautionary thinking.

The hands-free recommendation for mobile phone calls remains even though trends of glioma incidences do not provide support for an increasing risk caused by mobile phone radio wave exposure. However, observed biological effects and uncertainties regarding possible long-term effects justify caution.

The Swedish authorities' recommendation to generally limit exposure to low frequency magnetic fields, due to the observed increased incidence of childhood leukaemia close to power lines, still remains.

Need for further research

Despite the fact that no health risks with weak electromagnetic fields have been established today, the Authority considers that further research is important, in particular regarding long-term effects as the entire population is exposed. One key issue here is to further investigate the relationship between radio wave exposure and oxidative stress observed in animal studies and to establish whether and to what extent it may affect human health.

¹ Chapter 2 in the Swedish Environmental Code, see <https://www.government.se/legal-documents/2000/08/ds-200061/>

There is also a need to further investigate the observed decreased sperm counts, sperm viability and decreased serum testosterone due to radio wave exposure of testes in animal studies before any conclusions concerning the possible implications for human health can be drawn.

Since many studies report impact on cognitive functions due to the use of information technology, it is desirable to further investigate if this association to some extent depend on the resulted radio wave exposure. To be able to draw reliable conclusions on this issue, it is important to further develop and apply methods that have the ability to clearly discriminate between different causal relationships.

Wireless information technology is constantly evolving and new frequency ranges will be used. The fifth generation mobile telecommunication system (5G) will be installed all over the world within the next few years. Even though there is no established mechanism for affecting health from weak radio wave exposure there is need for more research covering the novel frequency domains used for 5G. The Authority also encourage researchers to start undertaking epidemiological studies, i.e. cohort studies, in this area.

New technologies for inductive wireless energy transfer based on intermediate frequency magnetic fields will probably be implemented for many different applications in the near future. In contrast to wireless information transfer technology, wireless energy transmission in principle always results in relatively strong local fields. This makes it very important to obtain a robust basis for risk assessment of such fields. Today there is a lack of studies in this frequency domain, therefore there is a special need for research.

Another vital issue to investigate is whether low frequency magnetic fields contribute to the increased incidence of childhood leukaemia that has been observed close to power lines in epidemiological studies.

It is also desirable to investigate different health effects based on combinations between electromagnetic fields and other factors, both physical factors and chemical factors.

There is also a need to better clarify the origin for the different problems MRI-exposed workers have experienced described in previous council reports.

Project information

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Recent Research on EMF and Health Risk

Fourteenth report from SSM's Scientific Council on Electromagnetic Fields, 2019

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Preface

The Swedish Radiation Safety Authority's scientific Council for electromagnetic fields (EMF) and health was established in 2002. The Council's main task is to follow and evaluate the scientific development and to give advice to the authority. In a series of annual reviews, the Council consecutively discusses and assesses relevant new data and put these in the context of available information. The result will be a gradually developing health risk assessment of exposure to EMF. The Council presented its first report in 2003. A brief overview of whether or how the evidence for health effects has changed over the first decade of reports was included in the eleventh report. The present report is number fourteen in the series and covers studies published from April 2018 up to and including December 2018.

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Stockholm in December 2019

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Executive Summary

This report reviews studies on electromagnetic fields (EMF) and health risks, published from April 2018 up to and including December 2018. The report is the fourteenth in a series of annual scientific reviews which consecutively discusses and assesses relevant new studies and put these in the context of available information. The result will be a gradually developing health risk assessment of exposure to EMF.

Static fields

Exposure to static (0 Hz) magnetic fields much greater than the natural geomagnetic field can occur close to industrial and medical/scientific equipment that uses direct current such as some welding equipment and various particle accelerators. The main sources of exposure to strong static magnetic fields (> 1 T)¹ are magnetic resonance imaging (MRI) devices for medical diagnostic purposes. Volunteer studies show that movement in such strong static fields can generate sensations such as vertigo and nausea. The thresholds for these sensations seem to vary considerably within the population. Personnel exposed to fields from MRI scanners can also be affected by these transient symptoms.

Epidemiology

Only one new study was identified and the conclusion from previous Council reports remains unchanged: Transient symptoms experienced by workers exposed to magnetic resonance imaging (MRI) scanners are well established, but there is a lack of knowledge regarding potential long-term health effects.

Human studies

The experimental study which was published in the reporting period observed no effects of static field exposure from two MRI scanners (1.5 T and 3.0 T) on sensory and pain perception as compared to sham exposure. This study adds a small piece of information to an area where there are still many open questions.

Animal studies

Exposure to static fields in experimental animals has not led to adverse health effects. Low static magnetic fields (0.8 mT) were reported to decrease oxidative stress in rats. Strong static magnetic field exposures of mice reduced radiation-induced lung damage at 1.5 T and induced vestibular-stimulated nystagmus at 4.7 T. Static electric fields with a strength in the range of the maximum ground level of ultra-high-voltage direct-current transmission lines (± 400 - ± 800 kV) showed no effects on haematological parameters in mice, but reversible effects on liver and hippocampal oxidative stress.

Cell studies

The results of the *in vitro* studies evaluated in this report confirm that static fields are able to induce only slight variations in the biological endpoints considered. A Chinese research group studied several endpoints such as mitochondrial potential, oxidative stress and ATP production. They do not provide a clear evidence for an effect of a specific exposure condition or on a specific cell type, although the results have been obtained on a large number of both healthy and cancer cells.

Extremely low frequency (ELF) fields

The exposure of the general public to extremely low frequency (ELF) fields (>0 Hz-300 Hz) is primarily from 50 and 60 Hz electric power lines and from electric devices and wiring in buildings.

¹ These magnetic fields (>1 T) are about a thousand times stronger than magnetic fields used in animal and cell studies which are most often in the mT-range. The geomagnetic field at the Earth's surface ranges from 25 to 65 microtesla (μ T). For comparison a fridge magnet has a strength of about 0.005 tesla when measured at close distance.

Regarding the exposure to ELF magnetic fields and the development of childhood leukaemia, the latest studies did not consistently observe an association. However, these studies did not use new approaches and the same limitations apply as in previous research. Thus, the conclusion from previous Council reports still holds: epidemiologically, associations have been observed, but a causal relationship has not been established.

Epidemiology

No new study on residential exposure to ELF magnetic fields and childhood leukaemia was published since the last SSM report. Also research on other outcomes in relation to ELF magnetic fields is scarce and does not provide new insights for health risk assessment.

Human studies

The number of studies continues to be very low with just one study identified in the current reporting period. The sporadic publications over the years address different endpoints, for example postural shift this year with other than the hypothesised results, EEG last year. There is no substantial new information on effects of extremely low frequency (ELF) fields from human experimental studies.

Animal studies

Similar to the previous Council reports, studies used exposure levels mostly in the 1 mT range and below at 50 or 60 Hz. The different studies described various and partly contradictory effects of ELF magnetic field exposure in rodents, but did not provide insight on potential ELF magnetic field mechanism(s). An environmental study on honey bees showed that ELF magnetic fields may be an environmental stressor for flying insects, having impact on their cognitive and motor abilities. However, this only underlines the absence of knowledge on biological-relevant mechanisms of ELF magnetic field exposure. A Zebrafish study addressing pigmentation may be of minor relevance; but Zebrafish studies are a useful tool addressing fertility, cardiovascular system etc. Analogously also round worm (*C. elegans*) is used in basic research. Finally, no study directly addressed possible mechanisms for childhood leukemia. But the increasing number of studies using the endpoint 'Cytokines' may indirectly address leukemia in the future.

Cell studies

As for the previous report, the ELF *in vitro* studies were carried out on different cell types, of healthy or cancer origin, and evaluated several biological endpoints, including proliferation, viability, antioxidant defences, epigenetics and DNA damage. The *in vitro* studies on ELF magnetic field exposure do not indicate induction of genotoxic and epigenetic effects, while it seems that oxidative stress is slightly induced. Concerning the other endpoints considered, results are not univocal, with increase, decrease or no differences when exposed samples were compared to sham controls.

Intermediate frequency (IF) fields

The intermediate frequency (IF) region of the electromagnetic spectrum (300 Hz-10 MHz) is defined as being between the extremely low frequency and the radiofrequency ranges. Despite increasing use of IF magnetic field-emitting sources such as induction hobs and anti-theft devices, scientific evaluation of potential health risks is scarce. For some of these sources, exposure assessment, especially of induced internal electric fields, remains challenging. The experimental studies on IF electromagnetic fields do not show any adverse health effects below current guidelines, but since there is only a very limited number of such studies available, no conclusions can be drawn at present. Additional studies would be important because human exposure to such fields is increasing, for example from different kinds of electronic article surveillance systems and the increasing use of induction cooking. Studies on possible effects associated with chronic exposure at low levels are particularly relevant for confirming adequacy of international exposure limits.

Epidemiology

Only one study has assessed potential risks from exposure to intermediate frequency fields. A re-evaluation of occupational IF magnetic field exposures in the INTERPHONE study did not provide evidence of an increased risk of glioma or meningioma among exposed workers.

Human studies

There is no new information concerning effects of exposure to intermediate frequency magnetic fields in humans.

Animal studies

Four mouse studies with exposures in the 7.5 kHz range did not result in adverse effects on genotoxicity, fertility, reproduction, learning or behaviour. It should be noted that the upper magnetic field strength (120 μ T) is about twice of nowadays cashiers' work place-exposures.

Cell studies

Only one study has been identified on the effects of IF magnetic field exposure on cell cultures, in the framework of the GERONIMO project. Like the few studies published in the previous years, no effects of IF alone have been detected. But in combination with two well-known genotoxic agents, co-exposures showed effects dependent on the experimental conditions applied.

Radiofrequency (RF) fields

The general public is exposed to radiofrequency fields (10 MHz-300 GHz) from different sources, such as radio and TV transmitters, Wi-Fi, cordless and mobile phones, base stations and wireless local area networks. Among parts of the public there is concern about possible health effects associated with exposure to radiofrequency fields. Measurements and exposure calculations have shown that a person's radiofrequency field exposure is dominated by personal mobile phone use. The exposure from environmental sources such as mobile phone base stations plays a minor role.

A topic of particular interest and growing concern with the public is the development of the fifth generation mobile telecommunication system, or 5G. This is intended to provide better service through higher data rates and faster response rates. The main concern is on the intention to use frequencies that are considerably higher than those currently used for the 3G and 4G systems. To date, however, the 5G technology is rolled out by using frequencies near those currently used by mobile telephony and Wi-Fi. The principal frequency band for this is around 3.5 GHz. In addition, frequency bands currently used for mobile phone will be used including a new 700 MHz frequency band. In order to provide the higher data rates and faster connection, frequency bands of around 26 GHz will be used in a more distant future, although these communication standards have not been defined yet. While quite a lot of scientific studies have been performed into possible effects of MHz frequencies only very few studies are available which have considered exposures to frequencies higher than about 6 GHz.

Electromagnetic fields at frequencies > 20 GHz are called millimetre waves and do not penetrate further than skin-deep in the body. This may be of relevance to take into account in future health evaluations. The Council will report on such studies as they become available.

Epidemiology

With respect to mobile phone use and brain tumours, various analyses of cancer incidence time trends did not observe patterns supporting the hypothesis of increasing incidence rates following, with some latency, the time period of mobile phone uptake. These new incidence studies demonstrate changes between diagnostic or topographic classification over time. For instance, the glioblastoma incidence has been increasing in the USA but at the same time other brain tumour diagnoses have decreased. As it is unlikely that radiofrequency EMF exposure from mobile phones is protective for some tumours and presents a risk for others, this rather indicates that such diverging trends are a result of changing coding praxis over time. Similarly, determining the location of tumours in the head has improved over

time due to improved imaging techniques, which in turn resulted in seemingly increasing rates of tumours at specified sites of the head, including lateral sites. In contrast, incidence of brain tumours with unknown location has decreased over time. Again, this is an indication of changes in diagnostic and coding praxis and not a consequence of mobile phone-related exposure. These new studies demonstrate that possible changes in coding praxis over time need to be considered in a meaningful manner when interpreting time trends of specific subgroup diagnoses.

A new study on mobile phone use and survival time of glioma patients in Sweden, Denmark and Finland did not observe that mobile phone users had a shorter survival time, which would indicate a cancer promoting effect of mobile phone radiofrequency EMF exposure. This finding is in contrast to a previous Swedish study by Carlberg and Hardell (2014). The new study, however, indicated that cases with a poor prognosis were less likely to start mobile phone use shortly prior to their diagnosis, probably due to already existing symptoms. This type of bias may also explain decreased odds ratios for regular users seen in the INTERPHONE papers.

New studies on mobile phone use and use of other electronic media, in relation to health-related quality of life, cognitive function and behaviour of children and adolescents, often report associations. Some studies point to other exposures related to media use but not radiofrequency EMF as a causal factor since the strongest associations were found with e.g. texting, which causes minimal amounts of exposure. These studies show that it is challenging to separate effects from radiofrequency EMF exposure from other aspects of mobile phone use such as being woken up during night, blue light exposure or addictive behaviour. This is especially the case when dealing with outcomes like health-related quality of life, cognitive functions or behaviour. A few attempts in this direction have been done and a Swiss study found indications for a radiofrequency EMF effect on cognitive functions. However, this observation needs to be confirmed in other populations applying a similar radiofrequency EMF dose approach. New studies on other outcomes than discussed were not very strong from a methodological perspective and no firm conclusions can be drawn.

Human studies

None of the four human experimental studies on radiofrequency EMF effects, which were published in the reporting period and which addressed various outcome parameters (electrodermal activity, heart rate variability, thermal pain threshold, and symptoms) did observe effects of exposure. These studies thus add evidence to the conclusion that there are no adverse short-term effects of radiofrequency EMF exposure.

Animal studies

The studies on the effects of radiofrequency EMF exposure on brain and behaviour showed inconsistent results. Several studies showed impairment of memory, while others, with virtually similar treatments, did not. For example, one study showed decreased exploratory activity, while in another study no effect on locomotor activity was found. In several studies no effects were observed on oxidative stress in the brain. Increased oxidative stress, however, was observed in the eye, testes and sciatic nerve, but not in kidney. In testes, radiofrequency EMF exposure resulted in decreased sperm counts and sperm viability and decreased serum testosterone. These results are in line with the results of animal studies discussed in the previous Council reports. There is a need for systematic reviews of these studies, in particular on the topics of oxidative stress and male fertility, before any conclusions concerning the possible implications for human health can be drawn.

Cell studies

The new *in vitro* studies confirm the previous Council conclusions that several endpoints have been investigated and in most cases no effect of the exposure was detected. Nevertheless, in some investigations, where high SAR values were considered, effects on some cellular parameters have been reported. As for the past years, several studies have been recognized but not considered, due to the scanty quality of the experimental set-up.

Sammanfattning

I rapporten granskas studier av elektromagnetiska fält och hälsorisker, publicerade från april 2018 till och med december 2018. Det är den fjortonde rapporten i en serie årliga vetenskapliga granskningar som fortlöpande diskuterar och utvärderar relevanta nya data och värderar dessa i förhållande till redan tillgänglig information. Granskningarna leder till en successivt förbättrad uppskattning av hälsorisker från exponering för elektromagnetiska fält.

Statiska fält

Exponering för statiska (0 Hz) magnetfält som är mycket starkare än det naturligt förekommande geomagnetiska fältet kan förekomma i närheten av industriell och medicinsk/vetenskaplig utrustning som använder likström, som t.ex. elsvetsutrustningar och olika typer av partikelacceleratorer. Den viktigaste källan till exponering för starka statiska magnetfält ($> 1 \text{ T}$)² är användningen av magnetkamera för medicinsk diagnostik. Studier på frivilliga försökspersoner har visat att rörelser i starka statiska fält kan inducera elektriska fält i kroppen och orsaka yrsel och illamående. Tröskelvärdena för dessa effekter tycks dock variera avsevärt mellan olika individer. Personal som exponeras för fält från magnetkameror kan påverkas av dessa övergående fenomen.

Epidemiologi

Endast en studie har identifierats under rapporteringsperioden och slutsatsen från rådets tidigare rapporter kvarstår: Övergående symptom hos personal som arbetar med magnetkamera är väl dokumenterade men kunskap om eventuella hälsoeffekter på längre sikt saknas.

Studier på människa

Den enda experimentella humanstudie som identifierats under rapporteringsperioden såg inte några effekter av exponering för statiska fält från två olika magnetkameror (1,5 T och 3,0 T) vad gäller smärta eller andra sensoriska förnimmelser. Studien tillför en liten pusselbit med information till ett område där kunskapsluckorna fortfarande är stora.

Djurstudier

Exponering av försöksdjur för statiska fält har inte visat på några skadliga hälsoeffekter. Statiska magnetfält på (0,8 mT) har rapporterats minska oxidativ stress hos råttor. Exponering av möss för starka statiska magnetfält minskade strålningsinducerade lungskador vid 1,5 T och orsakade nystagmus (ofrivilliga ögonrörelser) härrörande från det vestibulära systemet (det balanssinne som sitter i öronen) vid 4,7 T. Statiska elektriska fält med en styrka i samma storleksordning som de starkaste kraftledningarna (400 – 800 kV) orsakar vid markytan visade ingen påverkan på hematologiska parametrar hos möss, men visade reversibla effekter på lever och oxidativ stress i hippocampus.

Cellstudier

Resultaten från de *in vitro*-studier som utvärderats i rapporten bekräftar att statiska fält endast kan orsaka små variationer i de biologiska parametrar som studerats. En kinesisk forskargrupp har studerat flera olika utfall, som mitokondriell potential, oxidativ stress och ATP-produktion (ATP är adenosintrifosfat). Resultaten ger inga säkra belegg för några effekter, varken vid specifika exponeringsförhållanden eller för specifika celltyper trots att ett stort antal celltyper, både friska celler och cancerceller, har studerats.

² De statiska magnetfälten i en magnetkamera är större än 1 T vilket är omkring tusen gånger starkare än de magnetfält som normalt används i djurstudier och cellstudier som oftast är i milliteslaområdet. Det geomagnetiska fältet vid jordytan varierar mellan 25 och 65 mikrottesla. En vanlig kylskåpsmagnet har en styrka på cirka 0,005 Tesla (5 millitesla) om man mäter alldeles intill.

Lågfrekventa fält

Allmänheten exponeras för lågfrekventa fält (>0-300 Hz) i första hand från kraftledningarna med frekvenserna 50 och 60 Hz och från elektriska installationer och apparater i byggnader. När det gäller sambandet mellan exponering för lågfrekventa magnetfält och utvecklingen av barnleukemi visar de senaste årens studier inte entydigt på samband. Inga nya undersökningsmetoder har emellertid använts i dessa studier och de har därför samma begränsningar som tidigare forskning. Därför gäller fortfarande slutsatsen från Rådets tidigare rapporter: I epidemiologiska studier har samband observerats men något orsakssamband har inte kunnat fastställas.

Epidemiologi

Sedan Rådets föregående rapport har det inte publicerats någon studie om exponering för lågfrekventa magnetfält och barnleukemi. Forskning om andra hälsoeffekter saknas i stor utsträckning och bidrar inte till en förståelse av eventuella hälsorisker.

Studier på människa

Antalet identifierade experimentella humanstudier är fortfarande mycket litet, endast en studie under rapporteringsperioden. Det fåtal studier som publicerats under åren har studerat flera olika slutpunkter, i årets studie t.ex. balansrubbningsningar med något annorlunda resultat än man förväntat sig, förra året studerades EEG (elektroencefalografi). Det har inte kommit fram någon väsentlig ny information om påverkan från exponering för lågfrekventa fält i experimentella humanstudier.

Djurstudier

Liksom i föregående rapporter från Rådet har studierna oftast rört exponeringsnivåer i området 1mT och lägre vid frekvenserna 50 och 60 Hz. De olika studierna har rapporterat ett flertal och delvis motstridiga effekter från exponering av gnagare för lågfrekventa fält, men resultaten från studierna har inte lett till någon ny kunskap om möjliga mekanismer. En experimentell studie av honungsbin visade att lågfrekventa magnetfält skulle kunna utgöra en stressfaktor för flygande insekter, med möjlig påverkan på deras kognitiva förmåga liksom deras motoriska förmåga. Detta understryker emellertid bara de obefintliga kunskaperna om biologiskt relevanta mekanismer vid exponering för lågfrekventa fält. En studie som undersökte effekter på pigmentering hos zebrafiskar är förmodligen av mindre betydelse men studier på zebrafiskar är ett användbart verktyg när det gäller att undersöka fertilitet, hjärt-kärlsystem och liknande. Även rundmaskar (*C. elegans*) används i grundläggande forskning. Slutligen, inte någon studie berörde direkt möjliga mekanismer för uppkomst av barnleukemi, men det ökande antalet studier om cytokiner (en grupp proteiner och peptider vars funktion är att bära kemiska signaler) kan indirekt komma att beröra barnleukemi i framtiden.

Cellstudier

I de cellstudier som analyserades undersöktes, liksom i föregående EMF rapport, olika typer av celler, både friska celler och cancerceller, och olika biologiska slutpunkter utvärderades, som celltillväxt, cellöverlevnad, antioxidantförsvaret, epigenetik och DNA-skador. Cellstudier med exponering för lågfrekventa magnetfält tyder inte på induktion av några genotoxiska eller epigenetiska effekter samtidigt som det finns en svag ökning av oxidativ stress. För övriga slutpunkter som studerats är resultaten inte entydiga. Jämfört med oexponerade kontroller kunde både ökning, minskning eller inga förändringar konstateras.

Intermediära fält

Det intermediära frekvensområdet (300 Hz-10 MHz) av det elektromagnetiska spektret ligger definitionsmässigt mellan det lågfrekventa och det radiofrekventa områdena. Trots en ökande användning av apparater som medför exponering för intermediära fält, som t.ex. larvbågar i butiker och induktionsspisar, så har eventuella hälsorisker utvärderats endast i mycket liten utsträckning. Exponeringsuppskattningen, särskilt för inducerade elektriska fält i kroppen, är fortfarande en utmaning för den här typen av exponeringskällor. De experimentella studierna avseende exponering för intermediära fält visar inte på några skadliga hälsoeffekter men eftersom det endast finns ett

mycket begränsat antal studier tillgängliga kan inga slutsatser dras för närvarande. Fler studier skulle vara värdefulla eftersom människor exponeras för dessa fält i ökande grad, t.ex. från olika typer av utrustning för artikelövervakning och en ökande användning av induktionshällar för matlagning. Studier av möjliga effekter vid långvarig exponering för låga nivåer är särskilt betydelsefulla för att bekräfta tillförlitligheten i gällande rikt- och gränsvärden.

Epidemiologi

Bara en studie har uppskattat potentiella hälsorisker från exponering för intermediära fält. En förnyad utvärdering av yrkesexponering för intermediära fält i INTERPHONE-studien har inte gett några belegg för ökad risk för gliom eller meningiom hos exponerad personal.

Studier på människa

Ingen ny information har framkommit under rapporteringsperioden vad gäller effekter av exponering för intermediära fält.

Djurstudier

Fyra studier på möss med exponeringar i frekvensområdet 7,5 kHz visade inte några negativa effekter på genotoxicitet, fertilitet, fortplantning, inlärning eller beteende. Det bör noteras att den högsta magnetiska fältstyrkan (120 μ T) är ungefär dubbelt så hög som den yrkesexponering som kassapersonal kan utsättas för idag.

Cellstudier

Endast en studie har identifierats som undersökt eventuella effekter på cellkulturer av exponering för intermediära fält. Studierna har ingått i GERoNIMO-projektet. Liksom för de få projekt som har publicerats under de närmast föregående åren har inga effekter av enbart exponering för intermediära fält kunnat upptäckas. Däremot har man kunnat se vissa effekter när exponeringen kombinerats med två välkända genotoxiska ämnen.

Radiofrekventa fält

Allmänheten exponeras för radiofrekventa fält (10 MHz-300 GHz) från en mängd olika källor som radio- och TV-sändare, trådlösa telefoner och mobiltelefoner och deras respektive basstationer samt från trådlösa datornätverk. Delar av allmänheten känner oro för möjliga hälsoeffekter som skulle kunna orsakas av exponering för radiofrekventa fält. Mätningar och beräkningar har visat att de högsta exponeringsnivåerna orsakas av användning av egen mobiltelefon. Omgivningskällor som basstationer för mobiltelefoni spelar endast en mindre roll.

Ett område som väcker särskilt intresse och även en växande oro hos allmänheten är utvecklingen av den femte generationens telekommunikationssystem, även kallat 5G. Systemet är avsett att ge förhöjd effektivitet genom högre datahastigheter och kortare svarstider. Den mesta oron handlar om att systemet kommer att utnyttja frekvenser som ligger avsevärt högre än de som nu används för 3G och 4G. Inledningsvis använder emellertid 5G-teknologin frekvenser som ligger nära de som idag används för mobiltelefoni och Wi-Fi. Det huvudsakliga frekvensbandet ligger runt 3,5 GHz. Utöver det kommer frekvenser som för närvarande utnyttjas för mobiltelefoni att användas plus ett nytt 700 MHz frekvensband. För att möjliggöra höga datahastigheter och snabbare uppkoppling kommer frekvensband runt 26 GHz att användas längre fram, men dessa kommunikationsstandarder har ännu inte definierats. Medan en stor mängd vetenskapliga studier har genomförts rörande eventuella hälsoeffekter vid exponering för MHz-frekvenser så har endast mycket få studier undersökt konsekvenser från exponering för frekvenser högre än 6 GHz. Elektromagnetiska fält med frekvenser högre än 20 GHz, kallade millimetervågor, tränger väsentligen inte längre in i kroppen än huden. Detta faktum kan vara viktigt att ta i beaktande vid framtida utvärderingar av eventuella hälsoeffekter. Rådet kommer att rapportera om sådana studier när de blir tillgängliga.

Epidemiologi

När det gäller mobiltelefonanvändning och hjärntumörer så visar flera olika analyser av tidstrender för cancerincidens inte något stöd för hypotesen att antalet nya fall, efter en viss latenstid, skulle öka med ökad användning. Dessa nya incidensstudier visar på ändringar i diagnostik och tumörklassificering i olika länder. Till exempel har incidensen av glioblastom ökat i USA men samtidigt har andra hjärntumördiagnoser minskat. Eftersom det är mindre troligt att exponering för radiofrekventa fält från mobiltelefoner skulle skydda mot vissa tumörer och utgöra en förhöjd risk för andra så tyder dessa skilda trender snarare på att klassificeringen av tumörer har ändrats över tid. På liknande sätt så har möjligheterna att lokalisera tumörer i huvudet förbättrats med tiden tack vare bättre bildteknik vilket i sin tur har resulterat i ett skenbart ökat antal tumörer i vissa delar av huvudet, inklusive laterala lägen (sida av huvudet). Samtidigt har incidensen av tumörer med okänd lokalisering minskat. Även detta är en indikation på ändrad diagnostik och förändrad klassificeringspraxis och inte en konsekvens av mobiltelefonrelaterad exponering. Dessa nya studier visar att man måste ta hänsyn till förändringar i klassificeringspraxis när man utvärderar tidstrender för specifika undergrupper av tumörer.

En ny studie har undersökt eventuella samband mellan användning av mobiltelefon och överlevnadstid för gliompatienter i Sverige, Danmark och Finland. Resultaten tyder inte på att mobiltelefonanvändare har en kortare överlevnadstid, vilket skulle ha kunnat tyda på att exponering för radiofrekventa fält från mobiltelefoner främjat en cancerutveckling. Detta resultat motsäger en tidigare studie av Carlberg och Hardell (2014). Den nya studien tyder på att det är mindre troligt att patienter med dålig prognos börjar använda mobiltelefon strax innan de får sin diagnos, förmodligen på grund av redan existerande symtom. Denna typ av metodfel kan också förklara varför man ser sänkta oddskvoter för regelbundna användare i artiklar från INTERPHONE-studien.

Nyare studier om användning av mobiltelefon och andra elektroniska media och hälsorelaterad livskvalitet, kognitiv funktion och beteende hos barn och ungdomar rapporterar ofta samband. En del studier pekar på andra orsakande faktorer än de radiofrekventa fälten eftersom de starkaste sambanden har observerats t.ex. när man skickade och tog emot sms, vilket ger en mycket låg exponering för radiofrekventa fält. Dessa studier visar att det kan vara svårt att skilja effekter från exponering för radiofrekventa fält från annan påverkan vid användning av mobiltelefon som t.ex. störd nattsömn, exponering för blått ljus och ett beroendeframkallande beteende. Detta är särskilt fallet när det handlar om utfall som hälsorelaterad livskvalitet, kognitiva funktioner eller beteende. Ett fåtal försök att undersöka detta har gjorts och i en studie från Schweiz sågs indikationer på en effekt på kognitiva funktioner vid exponering för radiofrekventa fält. Detta fynd måste emellertid bekräftas hos andra grupper av personer med en liknande typ av exponering. Nya studier, av andra utfall än de diskuterade, var inte särskilt övertygande ur ett metodologiskt perspektiv och några säkra slutsatser kan inte dras.

Studier på människa

Fyra experimentella humanstudier av påverkan från exponering för radiofrekventa fält har identifierats under rapporteringsperioden. De har studerat olika utfall som förändring av det elektriska motståndet i huden, variationer i hjärtfrekvens, termisk smärtröskel och symtom. Inte i någon av dessa studier kunde några effekter av exponeringen observeras. Dessa studier förstärker slutsatsen att det inte finns några skadliga korttidseffekter av exponering för radiofrekventa fält under gällande riktvärden.

Djurstudier

Studier rörande effekter av exponering för radiofrekventa fält på hjärna och beteende visade motsägelsefulla resultat. Flera studier visade på nedsatt minnesfunktion, medan andra, med praktiskt taget samma behandling, inte visade på någon försämring. Till exempel visade en studie på sänkt förmåga till utforskande aktivitet, medan en annan studie inte fann någon påverkan på den lokomotoriska förmågan (sättet att röra sig). I flera studier observerades inte någon påverkan på oxidativ stress i hjärnan. Däremot observerades ökad oxidativ stress i öga, testiklar och ischiasnerv, men inte i njurar. I testiklar orsakade exponering för radiofrekventa fält ett minskat antal spermier, minskad överlevnadsförmåga hos spermier och sänkt testosteronhalt. Dessa resultat ligger i linje med resultaten från djurstudier som utvärderats i tidigare rapporter från Rådet. Det behövs en systematisk

genomgång av dessa studier, framför allt rörande oxidativ stress och manlig fertilitet, innan det går att dra några slutsatser om betydelsen för människors hälsa.

Cellstudier

Nya *in vitro*-studier bekräftar Rådets tidigare slutsats att flera slutpunkter har undersökts och att man i de flesta fall inte kunnat se några effekter av exponeringen. I några undersökningar, där höga SAR-värden kommit ifråga, har man dock rapporterat effekter för några cellparametrar. Under senare år har ett stort antal studier identifierats, men inte utvärderats av Rådet beroende på undermåliga försöksupplägg.

Preamble

In this preamble we explain the principles and methods that the Council uses to achieve its goals. Relevant research for electromagnetic fields (EMF) health risk assessment can be divided into broad sectors such as epidemiologic studies, experimental studies in humans and in animals, and *in vitro* studies. Studies on biophysical mechanisms, dosimetry, and exposure assessment are also considered as integrated parts in these broad sectors. A health risk assessment evaluates the evidence within each of these sectors and then weighs together the evidence across the sectors to provide a combined assessment. This combined assessment should address the question of whether or not a hazard exists, i.e. if a causal relation exists between exposure and some adverse health effect. The answer to this question is not necessarily a definitive yes or no, but may express the likelihood for the existence of a hazard. If such a hazard is judged to be present, the risk assessment should also address the magnitude of the effect and the shape of the exposure response function, i.e. the magnitude of the risk for various exposure levels and exposure patterns.

As a general rule, only articles that are published in English language peer-reviewed scientific journals³ since the previous report are considered by the Council. A main task is to evaluate and assess these articles and the scientific weight that is to be given to each of them. However, some of the studies are not included in the Council report either because the scope is not relevant, or because their scientific quality is insufficient. For example, poorly described exposures and missing unexposed (sham) controls are reasons for exclusion. Such studies are normally not commented upon in the annual Council reports (and not included in the reference list of the report)⁴. Systematic reviews and meta-analyses are mentioned and evaluated, whereas narrative and opinion reviews are generally not considered.

The Council considers it to be of importance to evaluate both positive and negative studies, i.e. studies indicating that exposure to electromagnetic fields has an effect and studies indicating a lack of an effect. In the case of positive studies the evaluation focuses on alternative factors that may explain the positive result. For instance, in epidemiological studies it is assessed with what degree of certainty it can be ruled out that an observed positive result is the result of bias, e.g. confounding or selection bias, or chance. In the case of negative studies it is assessed whether the lack of an observed effect might be the result of (masking) bias, e.g. because of too small exposure contrasts or too crude exposure measurements. It also has to be evaluated whether the lack of an observed effect is the result of chance, a possibility that is a particular problem in small studies with low statistical power. Obviously, the presence or absence of statistical significance is only one of many factors in this evaluation. Indeed, the evaluation considers a number of characteristics of the study. Some of these characteristics are rather general, such as study size, assessment of participation rate, level of exposure, and quality of exposure assessment. Particularly important aspects are the observed strength of the association and the internal consistency of the results including aspects such as exposure-response relation. Other characteristics are specific to the study in question and may involve aspects such as dosimetry, method for assessment of biological or health endpoint and the relevance of any experimental biological model used.⁵

It should be noted that the result of this process is not an assessment that a specific study is unequivocally negative or positive or whether it is accepted or rejected. Rather, the assessment will result in a weight that is given to the findings of a study. The evaluation of the individual studies within a sector of research is followed by the assessment of the overall strength of evidence from that sector with respect to a given outcome. This implies integrating the results from all relevant individual studies into a total assessment taking into account the observed magnitude of the effect and the quality of the studies.

³ Articles are primarily identified through searches in relevant scientific literature data bases; however, the searches will never give a complete list of published articles. Neither will the list of articles that do not fulfill quality criteria be complete.

⁴ Articles not taken into account due to insufficient scientific quality are listed in an appendix and reasons for not being taken into account are indicated.

⁵ For a further discussion of aspects of study quality, see for example the Preamble of the IARC (International Agency for Research on Cancer) Monograph Series (IARC, 2002).

In the final overall evaluation phase, the available evidence is integrated over the various sectors of research. This involves combining the existing relevant evidence on a particular endpoint from studies in humans, from animal models, from *in vitro studies*, and from other relevant areas. In this final integrative stage of evaluation the plausibility of the observed or hypothetical mechanism(s) of action and the evidence for that mechanism(s) have to be considered. The overall result of the integrative phase of evaluation, combining the degree of evidence from across epidemiology, human and animal experimental studies, *in vitro* and other data depends on how much weight is given on each line of evidence from different categories. Human epidemiology is, by definition, an essential and primordial source of evidence since it deals with real-life exposures under realistic conditions in the species of interest. The epidemiological data are, therefore, given the greatest weight in the overall evaluation stage. However, epidemiological data has to be supported by experimental studies to establish a causal link between exposure and health.

An example demonstrating some of the difficulties in making an overall assessment is the evaluation of ELF magnetic fields and their possible causal association with childhood leukaemia. It is widely agreed that epidemiology consistently demonstrates an association between ELF magnetic fields and an increased occurrence of childhood leukaemia. However, there is lack of support for a causal relation from observations in experimental models and a plausible biophysical mechanism of action is missing. This had led the International Agency for Research on Cancer (IARC) to the overall evaluation of ELF magnetic fields as “possibly carcinogenic to humans” (Group 2B).

1. Static fields

1.1. Epidemiological studies

The latest Council reports concluded that transient symptoms experienced by workers exposed to magnetic resonance imaging (MRI) scanners were well established, but there was a lack of knowledge regarding potential long-term health effects.

1.1.1. Geomagnetic field and myocardial infarction

The Earth's geomagnetic field exhibits seasonal variation in strength and frequency composition. Jarusevicius et al. (2018) used data from the Lithuanian magnetometer to investigate correlations between these natural fluctuations and incidence of myocardial infarction (MI) in 435 male and 268 female persons hospitalized at the University Hospital of the Lithuanian University of Health Sciences during the year 2016. They observed various significant negative correlations between MI and mean weekly magnetic field strength for different frequency bands between 0 and 65Hz with correlation coefficients around -0.25. In women, there was a positive correlation coefficient of 0.25 ($p=0.037$) for the 2-65 Hz frequency range. When analysing data separately for the first and second half of the year, significant associations were largely confined to the first half of the year.

No explanation is offered for the different results according to half of year or why this analysis was performed in the first place; and neither ambient temperature, air pollution, nor any other potential confounders are accounted for, which altogether impedes a causal interpretation of this study.

1.1.2. Conclusions on static field epidemiological studies

Since the previous report, no new report regarding possible health effects from MRI has been identified. The conclusion from the latest reports therefore remains unchanged: Transient symptoms experienced by workers exposed to magnetic resonance imaging (MRI) scanners are well established, but there is a lack of knowledge regarding potential long-term health effects.

1.2. Human studies

Overall there is a lack of experimental human studies investigating health or biological effects of static fields (see review by Petri et al. (2017), which was discussed in the previous Council report). While there were no studies of sufficient quality published in the previous reporting reports, the current reporting period contributed one (single-blind) study.

In a placebo-controlled randomized study Kamm et al. (2019) investigated whether static magnetic field exposure affects sensory (touch) and pain perception (thresholds from three modalities: pinprick, pressure, and heat). They exposed 18 young healthy right-handed subjects (23.1 ± 1.8 years; 50 % females) to three different field strengths: 0 T (sham condition), 1.5 T and 3 T in clinical MRI scanners on three separate days (mean time interval between sessions: 1 - 34 days). Exposures lasted 10 min each and were applied in a randomized order, however, only the subjects were blinded to the exposure condition (single-blind study design). The sensory and pain testing was performed immediately before and after each magnetic field exposure. The duration of each testing was maximum 15 min. None of the outcome parameters was affected by the static magnetic field, except skin temperature which was significantly lowest in the sham condition and highest (difference 0.8 °C) in the 3 T exposure condition.

1.2.1. Conclusions on static field human studies

The experimental study, which was published in the reporting period observed no effects of static field exposure from two MR scanners (1.5 T and 3.0 T) on sensory and pain perception as compared to sham. This study adds a small piece of information to an area where there are still many open questions.

1.3. Animal studies

In contrast to the previous Council report with only two experimental studies, six studies on static field effects were found. Two studies addressed effects on brain and behaviour, one on haematological parameters; another three addressed the effect of static field exposure on oxidative stress markers, and on radiation-induced lung injury. In three studies of the total six, a Chinese research group (Di et al. (2018), Lin et al. (2018), Xu et al. (2018)) evaluated potential health effects of ultra-high-voltage-direct current transmission in ICR mice.

1.3.1. Brain and behaviour

Ward et al. (2018) exposed 8 C57BL/6J mice and 6 head tilt mice (= B6.129S1-Nox3het-3J/GrnrJ mice lacking in Nox3 gene, “which is required for normal otoconial development”) to 4.7 T. Before that, 4.7 T magnetic vestibular stimulation (MVS) and vestibulo-ocular reflexes of the mice were measured using videoculography. Outside the magnet and during whole-body sinusoidal rotations and tilts, both mouse strains had intact horizontal vestibulo-ocular reflex, but only C57BL/6J mice exhibited static counter-roll responses to tilt (normal utriculo-ocular reflex). Following 4.7 T static magnetic field application, C57BL/6J mice had left-beating nystagmus of 32.8 s duration (median), when placed in the magnet nose-first. After tail-first entry into the magnet bore, the direction reversed (nystagmus was right-beating) but of similar duration (median 28.0 s). Head tilt mice lacked magnetic field-induced nystagmus. In conclusion, 4.7 T MVS led to nystagmus in intact (with a normal utricle) C57BL/6J mice, but not in mice deficient in Nox3.

Xu et al. (2018) evaluated the short term (7 days) and long term (49 days) effect of 56.3 kV/m static electric field (SEF) on learning and memory in mice. Four groups of n=10 male 4-week-old ICR mice were used: (1) 7 d exposure, (2) 7 d sham, (3) 49 d exposure, (4) 49 d sham. Morris water maze (MWM) tests were performed on days 2-6 for groups (1) and (2) and on days 44-48 for groups (3) and (4). Following 7 days short-term SEF exposure, the escape latency was significantly prolonged, the number of platform-site crossovers and the time spent in the target quadrant in the MWM test were decreased. In the hippocampus, serotonin (5-HT) level was increased and the ratio of glutamate level to γ -aminobutyric acid level (Glu/GABA) significantly decreased. Malondialdehyde (MDA) content and glutathione peroxidase GSH-PX activity increased significantly, while superoxide dismutase activity (SOD) decreased significantly. By contrast, after 49 days long-term SEF exposure, no significant differences of the above parameters between the SEF and sham exposure groups were observed. Summarizing, short term exposure to 56.3 kV/m static electric fields changed neurotransmitter levels and oxidative stress in the hippocampus, which corresponded with reduced learning and memory ability. After long-term exposure, the above SEF-induced disturbances returned to normal, i.e., the effects of 56.3 kV/m SEF was reversible and duration-dependent.

1.3.2. Physiology, pathophysiology and oxidative stress

Coballase-Urrutia et al. (2018) tested different oxidative stress markers in restraint Wistar rats following whole-body exposure to 0.8 mT static magnetic field (SMF) exposure and sham exposure of 30 min, 1 h or 2 h/d for 5 consecutive days. Seven groups with n=8 rats each were used: (1) cage control, (2) 30 min restraint, (3) 1 h restraint, (4) 2 h restraint, (5) 30 min restraint + SMF, (6) 1 h restraint + SMF, (7) 2 h restraint +SMF. After 5 days of exposure, blood plasma samples were taken.

Compared to control (1), in restrained rats an increase of NO (nitric oxide), MDA (malondialdehyde), AOPP (advanced oxidation protein products), and decreased SOD (superoxide dismutase), GSH (glutathione), and AGEs (glycation end products) were found. The response to restraint stress was reduced over time (30 min, 1 h, 2 h) for NO, SOD, AOPP, GSH, AGEs, but increased for MDA. Compared to the respective sham controls (2, 3, 4), the additional SMF exposure (5, 6, 7) resulted in time-dependent (30 min, 1 h, 2 h) decreased levels of NO, MDA, AGEs and AOPP, whereas SOD and GSH were increased. The authors summarized that the response to SMFs was time-dependent. This is confusing since the time-dependencies of the sham-restraint rats were not addressed. Additional inaccuracies are: Age and sex of the rats are not specified, the SMF was measured with a gasometer over 1 year, figure 3 in the study should present MDA results, but GSH is written and the bars of figure 3 and figure 4 are identical. Finally, by placing ferromagnetite magnets like shields at both sides of the restraint boxes, the SMF-exposed rats may feel more comfortable than the sham controls (only tube-restrained); i.e., SMF-exposed rats may have been less stressed. Overall the authors' conclusion that exposure to weak-intensity SMFs could offer a complementary therapy by attenuation oxidative stress is questionable.

Di et al. (2018) compared effects of 35 kV/m static electric field (0 Hz, SEF) and 35 kV/m ELF-EF (50 Hz) on haematological parameters in mice. [The ELF-EF part of the study is described in chapter 2.3.4.] Groups of n=10 male 4-week old ICR mice were continuously exposed to SEF for 7, 14, and 21 days, another n=10 mice/time point served as non-exposed controls. (The exposure unit was the same as used by Wu et al. (2017); compare 13th Council report (2019).) Following 7, 14 or 21 days of SEF-exposure, blood was taken, and the following parameters determined: White and red blood cell count (WBC and RBC), haemoglobin concentration (Hb), differential blood count (NE%, LYM%, MO%, EO%, BAS%). Compared to non-exposed control mice, the exposure to 35 kV/m static electric field did not alter the above blood parameters.

Rubinstein et al. (2018) investigated a potential effect of a 1.5 T MF on radiation-induced lung damage in mice. Groups of twenty 8-week-old female C57BL/6J mice were whole thorax-irradiated (Co-60) to doses of 0, 9.0, 10.0, 10.5, 11.0, 12.0, or 13.0 Gy. Simultaneously and transverse to the radiation beam, ten mice per group received 1.5 T. The other 10 mice were sham-exposed (0 T MF). Survival was checked daily, non-invasive assays of lung damage (respiratory rate (RR), lung density (LD), and lung volume (LV)) monthly until study termination at 8 months post-irradiation. All mice of 0, 9.0, 10.0 Gy groups survived while the survival rate of 10.5 – 13.0 Gy groups decreased dose-dependently. Overall, the simultaneously applied MF of 1.5 T had no impact on survival, but 1.5 T had a small statistically significant effect on radiation-induced lung damage; compared to 0 T (sham) in 1.5 T mice the ED50 was 2% lower for RR and LV, and 3% lower for LD. Since similar to humans, C57BL/6 mice are susceptible to acute pneumonitis and subsequent chronic fibrosis at post-irradiation time-points, a responsible clinical use of MRI-guided radiation therapy systems (MRIgRT) must be ensured.

Lin et al. (2018) aimed to examine in a further experiment (compare 13th Council report, SSM 2019, Wu et al. (2017)) whether static electric field (SEF) induce health risks in liver. Male 4-week old ICR mice were exposed up to 35 days (24 h/d) to SEF-intensities of 27.5, 34.7 and 56.3 kV/m. A sham-exposure group corresponded to each SEF-intensity. Liver function (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) and oxidative stress (superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA)) were tested after exposure of 7, 14, 21 and 35 days. SEF of 27.5 kV/m and 34.7 kV/m for 35 days did not alter the above parameters of liver function and oxidative stress, whereas the SEF-intensity of 56.3 kV/m significantly increased the liver SOD-activity after exposure for 7 and 14 days. But no (significant) increase was found in AST-, ALT-activities and in MDA content between 56.3 kV/m SEF and sham-exposure group. The authors discuss that this biological effect may be related to the increase of mitochondrial membrane potential of hepatocytes caused by SEF exposure. When the membrane potential exceeds a threshold, Q cycle in mitochondria will be affected, which will result in an increase of superoxide anion concentration and ultimately an oxidative stress. In conclusion, only exposure to the highest intensity of 56.3 kV/m SEF for a short time (7 and 14 days) could induce a certain oxidative stress response in the liver of mice but

did not cause an obvious oxidative damage. Finally, the authors suggest for future studies larger sample sizes and an exposure time much longer than 35 days.

1.3.3. Summary and conclusions on static magnetic and electric field animal studies

Low static magnetic fields were reported to decrease oxidative stress in rats (0.8 mT). Strong static magnetic field exposures reduced radiation-induced lung damage in mice (1.5 T) and induced vestibular-stimulated nystagmus in mice (4.7 T). Finally, static electric fields with a strength in the range of the maximum ground level of UHDV (ultra-high-voltage direct-current of $\pm 400 - \pm 800$ kV) transmission lines showed no effects on haematological parameters, but reversible effects on hippocampal oxidative stress and neurotransmitter contents in mice.

Table 1.3.1. Animal studies on exposure to static magnetic fields

Endpoint in rodents	Reference	Exposure SMF / SEF	Exposure Duration and Species	Effect
Brain and behaviour	Ward et al. (2018)	4.7 T	≥ 1 min Mouse	C57BL/6J mice generate nystagmus, Nox3-deficient (head tilt mice) do not.
	Xu et al. (2018)	56.3 kV/m	7, 49d 24 h/d? Mouse	7d: Learning & memory declined, hippocampal serotonin and oxidative stress increased. 49d: No differences to sham control.
(Patho)Physiology & Oxidative Stress	Coballase-Urrutia et al. (2018)	0.8 mT	5d 30min/d, 1h/d, 2h/d Rat	SMF may decrease oxidative stress in restraint rats
	Di et al. (2018)	35 kV/m	7, 14, 21d 24h/d Mouse	No differences in hematology (vs. non-exposed control).
	Lin et al. (2018)	27.7, 34.7, 56.3 kV/m	7, 14, 21, 35d 24h/d Mouse	SOD activity in liver increased after 7 and 14 d only, i.e. temporary stress response in liver.
	Rubinstein et al. (2018)	1.5 T + 0, 9, 10, 10.5, 11, 12, 13 Gy simultaneously	Once acute Mouse	No influence on survival. Respiratory rate, lung density and lung volume decreased.

1.4. Cell studies

Among the 12 studies found in the period of interest, six have not been included in the analysis due to scanty quality of the research. The six studies considered addressed the effect of exposure on cell metabolic status, oxidative stress, apoptosis, cell growth and DNA integrity.

Glinka et al. (2018) evaluated the effect of SMF (static magnetic field) of different intensities on cell redox and metabolic status of primary cultures of fibroblasts isolated from tails and belly skin of 60-day-old mice. The exposure was 72 h long in patented home-made exposure chambers, designed and realized to have six magnetic flux densities ranging from about 0.3 to 0.7 T by changing the thickness of the permanent magnet.

The results of five independent experiments indicated no differences between sham-exposed and MF-exposed cultures in glutathione reductase (Gr) activity, malondialdehyde (MDA) generation, ATP concentrations as well as total antioxidant status (TAS). At variance, a decrease in the activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) was recorded for all the flux densities investigated ($p < 0.05$). These results indicate that the experimental conditions adopted do not cause oxidative stress in exposed fibroblasts, but a slight antioxidant activity is induced.

Kimsa-Dudek et al. (2018) employed a normal human dermal fibroblasts (NHDF) cell line to investigate the effects of SMF in presence and in absence of Fluoride, a cytotoxic agent inducing apoptosis, oxidative stress, general changes in DNA and RNA and protein biosynthesis, on intracellular reactive oxygen species (ROS) production and on the expression profile of the genes associated with the antioxidant the defence system. SMF was delivered for 24 hours at a magnetic flux density of 0.65 T. The results of three independent experiments indicated that the exposure to SMF alone did not affect ROS production, while fluoride ions induced ROS generation, as expected. The combined exposure resulted in a significantly reduced fluoride-induced ROS production ($p < 0.05$). In addition, co-exposure to SMF restored the expression of the genes altered by fluoride.

Three papers have been published by a research group from Hefei, China.

In a first study Tian et al. (2018) investigated the effect of 48 h exposure to SMF on 12 different cell types by testing cell growth under different field directions and intensities. Permanent magnets were used to obtain 0.2-1 T field intensities. For each condition the authors carried out three independent experiments in blind. The results indicated that, by comparing sham-exposed and exposed cultures, an upward MF of 0.2-1T reduced the cell number of all the human solid, adherent, cancer cell lines ($p < 0.05$) but no effects were detected following exposure to a downward MF. The leukaemia cells in suspension were inhibited by both upward and downward MF. In contrast, healthy cell lines did not exhibit any effect under MF exposure in any of the directions. Magnetic resonance imaging (MRI) machines have horizontal or upright static magnetic field of 0.1–3 T at sites of patients and operators. The authors stated that their results verified the safety of SMF exposure related to current MRI machines and suggest a possible antitumor potential of MF with an upward direction.

In a second study Wang et al. (2018a) tested the effect of moderate and strong SMFs on ATP level and mitochondrial membrane potential (MMP) on 13 cell lines. In particular, 8 human cancer cell lines, 3 rat cancer cell lines and 2 non-cancer cell lines. The basic experiments were conducted on PC12 (adrenal gland rat cancer cells). Cultures were exposed or sham-exposed for 6 h to 0.26, 0.5, 1 and 1.13 T. SMFs of 1 and 1.13 T induced a significant increase in ATP level ($p < 0.05$) while 0.26 and 0.5 T did not. On the same cell types, the authors also tested different exposure duration (1, 3, 6 and 9 h) at 1 T; in this case ATP level and MMP were evaluated. Following 1 and 6 h exposure a statistically significant increase in both parameters was detected ($p < 0.05$) while no effects were found following 3 and 9 h exposure. When 3 and 6 h exposures at 9 T were investigated, a decrease in both parameters was detected only at 3 h ($p < 0.05$). To test whether the results obtained were specific for PC12 cell lines, 12 other cell types were employed to measure ATP level following 6 h exposure at 0.26, 0.5 and 1 T and following 3 and 6 h exposure at 9 T. The results indicated an increased ATP level vs. sham controls only after 6 h at 1 T in 6 out of 12 cell types tested. Exposures to 9 T resulted in an ATP decrease at 3 h exposure on 6 out of 12 cell types tested and an increase after 6 h exposure in 2 out of 12 cell types tested ($p < 0.05$; $n=3$).

In another paper, the authors tested the effect of 2, 4 and 6 h exposure to 6 mT on differentiated and undifferentiated PC12 cell line (Wang et al., 2018b). The results of three independent experiments indicated no effect on ATP level in both cell types. To evaluate if such effect is cell type-dependent, nine more cell lines, such as five human cancer cell lines (HeLa, HCT116, MCF7, A549, and GIST-T1), a rat cancer cell line (C6), and three non-cancer cell lines (RPE1, CHO, and 293T), were employed to test the ATP level after 2, 4 and 6 h exposure. A slight increase was detected in some but not in all the cells tested at some but not all the exposure durations.

In addition, the levels of mitochondrial membrane potential (MMP) and of ROS were measured in HTC116, 293T, GIST-T1 and RPE1 cells following 2 h exposure. No effects were detected except for a decrease in MMP level in GIST-T1 cells and an increase in RPE1 cells ($p < 0.05$). ROS levels resulted increased in HCT 116 cells and decreased in 293T cells ($p < 0.05$).

Although such results have been obtained on a large number of cell types, they do not provide a clear evidence for an effect on a specific cell type. In this paper, the effect of exposure to ELF fields was also investigated and the results are reported in section 2.4.

Yuan et al. (2018) used Human nephroblastoma G401, mouse neuroblastoma N2a and Human neuroblastoma CHLA255 cell lines to evaluate the number of live cells after exposure to a SMF, 5.1 mT field intensity, given 2 h/day for 1, 2 and 3 days. In three independent experiments a reduced cell number was detected after 2 and 3 days exposure in all the cell types investigated ($p < 0.05$).

1.4.1. Summary and conclusions for cell studies

The results of the studies considered confirm that static fields are able to induce only slight variations in the biological endpoints considered. Concerning the results reported by the research group from Hefei, China, although they have been obtained on a large number of healthy and cancer cells, they do not provide a clear evidence for an effect on a specific exposure conditions or on a specific cell type.

Table 1.4.1. Cell studies on exposure to static magnetic fields

Cell type	Endpoint	Exposure conditions	Effect	References
Mice primary fibroblasts	cell redox and metabolic status	0.3 – 0.7 T 72 h	No variation in Gr activity, MDA generation, ATP concentration and total antioxidant status. Decreased SOD and GPx activity.	Glinka et al (2018)
Normal human dermal fibroblasts (NHDF)	apoptosis, oxidative stress, DNA, RNA, protein biosynthesis	0.65 T 24 h Co-exposure with Fluoride	No effects of SMF alone. Fluoride-induced ROS formation and gene expression alteration was reduced by co-exposure to SMF.	Kimsa-Dudek et al (2018)
12 different cell types: 6 cancer and 6 healthy	Cell growth	0.2-1 T Upward and downward MF 48 h	Reduction in cancer cells under upward MF; No effect of downward MF. No effects in healthy cell lines.	Tian et al (2018)
13 different cell types: 8 human cancer, 3 rat cancer and 2 non-cancer cell lines.	ATP level and MMP	0.26, 0.5, 1, 1.13 and 9 T 1, 3, 6, 9 h	Increase, decrease or no effects on one or both parameters on the bases of the exposure conditions and of the cell type investigated.	Wang et al (2018a)
10 different cell types: 5 human cancer, 2 rat cancer and 3 non-cancer cell lines.	ATP level, MMP and ROS formation	6 mT 2, 4, 6 h	Increase, decrease or no effects on one or more parameters on the bases of the exposure conditions and of the cell type investigated.	Wang et al. (2018b)
Human nephroblastoma G401; mouse neuroblastoma N2a; Human neuroblastoma CHLA255	Cell viability	5.1 mT 2 h/day for 1, 2 and 3 days	Reduced cell number after 2 and 3 days exposure in all cell types	Yuan et al (2018)

Abbreviations: ATP: adenosine triphosphate; GPx: glutathione peroxidase; Gr: glutathione reductase; MDA: malondialdehyde; MMP: mitochondrial membrane potential; ROS: Reactive oxygen species; SMF: static magnetic fields; SOD: superoxide dismutase.

2. Extremely low frequency (ELF) fields

2.1. Epidemiological studies

In the previous Council reports (SSM 2015, SSM 2016, SSM 2018) it was concluded that little progress had been made to resolve whether the consistently observed association between ELF magnetic fields (ELF-MF) exposure and childhood leukaemia in epidemiology was causal or not. Another open question was related to occupational ELF-MF exposure and/or electric shocks as a risk factor for amyotrophic lateral sclerosis (ALS) and Alzheimer diseases (AD). Although associations are often reported, there is no consistent pattern that suggests either ELF-MF, electric shock or both factors together as the cause. It was also noted that little research was conducted on other outcomes in relation to ELF-MF exposure.

2.1.1. Childhood cancer

Su et al. (2018b) meta-analysed 22 studies that had evaluated parental occupational ELF-MF exposure in association with childhood nervous system tumours but not neuroblastoma. Maternal, but not paternal, exposure appeared to be associated with observed increased risks, with an OR of 1.16 (1.06–1.26) and moderate heterogeneity between studies ($I^2=44\%$). The authors concluded that better exposure and outcome assessment would be necessary in future studies to assess such associations.

A small case-control study on parental occupational exposures and childhood leukaemia was published by Kyriakopoulou et al. (2018). They included visiting and hospitalised cases due to ALL (86%) and AML (14%) and invited controls visiting the hospital for acute conditions but not malignancies. Cases and controls were matched based on age of the child at diagnosis, gender and ethnicity. 108 cases and the same number of controls were included into the study; participation rate was reported to be 97%. Parental occupations held for more than 6 months were inquired by means of a questionnaire, coded into ISCO08, and occupational exposures to different agents including EMF was assigned to the codes, based on knowledge from literature and experience from occupational hygienists. Mothers were reported to have been occupationally unexposed to EMF, but 6 cases as well as 6 control fathers had had occupational EMF exposure one year before conception, which translated to a crude OR of 1.49 (95% CI 0.42-5.53).

This is a very small study, and unfortunately, the authors did not explain if the EMF classification included static, extremely-low frequency or radiofrequency fields. Regarding paternal EMF exposure and possible effect on leukaemia in offspring, the study is uninformative.

2.1.2. Neurodegenerative diseases

Roosli and Jalilian (2018) meta-analysed residential exposure to ELF-MF and risk of amyotrophic lateral sclerosis. Five studies were found and risks were pooled across participants who either lived within 200 m of a power line or who were exposed to levels above 0.1 μ T. No increased risks were observed, but the number of high exposed cases was very low and it is therefore unclear if this risk estimate may be interpreted as evidence for an absence of risk.

Occupational ELF-MF exposures and risk of amyotrophic lateral sclerosis (ALS) were systematically reviewed and meta-analysed by Gunnarsson and Bodin (2018). Eleven studies that were deemed to be of sufficiently high quality were meta-analysed and resulted in a statistically elevated summary risk estimate of 1.23 (95% CI 1.04-1.45). As such, this meta-analysis is in line with previous systematic reviews that concluded a slight increased risk for ALS among exposed workers.

Checkoway et al. (2018) compared prevalence of Parkinsonism symptoms among 573 former textile workers and compared them to symptoms in a referent worker group (N= 286, birth year strata

frequency matched), who had never worked in the cotton textile industry. Participation rate was 49% and 22% among the two groups, respectively. Participants underwent Mini-Mental and neurological tests including assessment of the cardinal signs of Parkinson's disease. In total, 669 persons (including 26 cases) underwent a repeat neurological exam 2.5 years after baseline. Occupational exposure to magnetic fields was expressed as microtesla-years, and the author's additionally explored exposures included endotoxin and night shift work. Magnetic field exposure was evaluated in groups (tertiles). Adjusted for age, smoking, neurologist and the other occupational exposures, the risk estimate among the highest group of microtesla-year exposed women ($>108 \mu\text{T}$ -years) was slightly elevated but not statistically significant with a Prevalence Ratio (PR) of 1.93 (0.67, 5.60); the PR for the second highest exposure group was below unity.

Weaknesses of the study include the relatively low sample sizes, low participation rate and some potential for healthy worker survival bias, as most participants were assessed about 20 years after retirement and residual confounding from potential co-exposures in the cotton textile industry (e.g. pesticides). Nevertheless, the result is in line with earlier meta-analyses that suggested that extremely-low frequency magnetic field exposure does not seem to be associated to Parkinson's disease or Parkinsonism risk.

2.1.3. Other outcomes

In a cross-sectional study, Shaifudin et al (2018) used Kolmogorov-Simonov tests to compare micronuclei frequencies from buccal samples in 128 children from two schools in Malaysia; one situated within 50 m and one more than 4 000 m from an overhead power line. No response-rate was provided. The mean field strengths assessed with spot measurements, using EMDEX IIs at the two schools were $0.112 \mu\text{T}$ and $0.027 \mu\text{T}$, respectively. The median micronuclei frequency among children at the school far from an overhead power line was significantly ($p<0.001$) higher than at the school close to the power line. The authors accounted for a range of individual factors such as second hand smoke and other sources of MF-exposure and concluded that the magnetic field-levels at the exposed school posed no health hazard.

The study was rendered largely uninformative as only two schools were compared making it impossible to account for other environmental factors that may differ between schools. Also lifestyle factors may differ, but no such confounders were considered in the analysis.

Bagheri Hosseinabadi et al. (2019) performed a cross-sectional study among workers of three substations and a control building in a petrochemical power plant in southern Iran. Of the workers, 132 were grouped as "exposed" and a further 143 workers as "unexposed". Two-minute spot measurements were performed and, based on reports of workers' usual work schedules and locations, 8-hour time-weighted average exposures was assigned to workers. Participants filled in the Pittsburgh questionnaire to assess sleep quality and another questionnaire was used to assess depression, stress and anxiety. Average exposures were reported to be $29 \mu\text{T}$ (SD $28 \mu\text{T}$). Technicians and operators were more likely to have higher exposure compared to office workers. The authors report linear associations of higher exposure levels with higher levels of stress, anxiety and depression. Unfortunately, the authors did not present any further explanation as to classification of workers into exposed or unexposed groups. Given that exposures were quite different per occupational group within the power plant, an alternative explanation (rather than a causal association with magnetic field exposure) includes different work practices that may be underlying the finding of higher stress, anxiety and depression scores among higher exposed workers.

2.1.4. Conclusions on ELF epidemiological studies

Recent studies do not alter the current interpretation of the observed association of residential exposure to ELF-MF and childhood leukaemia with no causal explanation. Research on other outcomes including neurodegenerative disease is scarce and does not provide new insights for health risk assessment.

2.2. Human studies

The number of human experimental studies on effects of extremely low frequency (ELF) fields continues to be very low. As in the previous reporting periods there is just one new study. It is from the Canadian group, which in the past years published several papers in which effects of ELF fields on various endpoints have been analysed. The present paper by Villard et al. (2019) addresses effects of extremely low-frequency (< 300 Hz) magnetic fields (MF) on acute standing balance responses, which was measured by a device to record centre of pressure displacement (COP) with a sampling rate of 10 kHz. Additionally, effects of stimulation by electric currents delivered by electrodes at the surface of the head were investigated. An AC electric current was used for comparison to ELF-MF exposure and DC stimulation was introduced as a positive control. It is known that DC currents can lead to a slight shift in posture. Twenty-two young healthy participants (23 ± 4.8 years, 10 females) were exposed. All stimulations were applied in one session. After explanation and obtaining informed consent, the set-up of the stimulation system and familiarization with exposures the actual test session started. It consisted of 33 randomized conditions split up into 3 sub-sessions with 11 testing conditions each. The sub-sessions lasted for 25 min each and were separated by a 5 min break. The 33 randomized exposures consisted of 1 sham exposure, 2 DC-GVS 1.5 mA exposures (GVS: galvanic vestibular stimulation with the anode at the left and right side, respectively), 10 AC-GVS 1.5 mA exposures (5 left side and 5 right side with stimulation frequencies of interest at: 20, 60, 90, 120 and 160 Hz), and 20 MF exposures (same 5 frequencies of interest applied on the left and on the right side and at 2 flux density levels 50 mT and 100 mT). Outcome parameters were the path length, the area, the velocity, and the power spectra in low (< 0.5 Hz) and medium (0.5 – 2 Hz) frequency bands of postural modulation. The direct current (DC) electric stimulation was used as positive control. As expected, effects were observed for the DC control, but not for the time-varying stimulations (AC and MF). The authors observed a significant stabilization effect of the custom experimental apparatus on postural data in the experiments. They argue that this might have neutralized hypothesized effects.

2.2.1. Conclusions on human studies

The number of studies continued to be very low (just one study in the current reporting period) and the sporadic publications over the years address different endpoints (e.g. postural shift this year with other than the hypothesised results, EEG last year). There is no substantial new information on effects of extremely low frequency (ELF) fields from human experimental studies.

2.3. Animal studies

During this reporting period, in total thirteen studies on brain and behaviour, oxidative stress, cytokines and miRNA, physiology and reproduction & development were identified in rodents. Finally, three studies in non-mammalians describe effects of power line MFs on honey bees, of ELF-MF on round worm (*C. elegans*), and of Pulsed ELF MF (PEMF) on pigmentation in Zebra fish.

2.3.1. Brain and behaviour

Zuo et al. (2018) used their previously established Alzheimer's disease (AD) rat model; see 11th Council report [(SSM, 2016), (Liu et al., 2015)]. In brief, premature aging and learning-memory disorder in that AD rat model is provided by daily intraperitoneal (ip) injection of D-galactose for 42 days + stereotactic hippocampal microinjection of A β 25-35 peptide fragments on day 43. D-galactose induces premature aging, A β 25-35 AD-like symptoms. Male Wistar rats of non-AD groups receive saline during similar procedures. After Morris water maze (MWM) training, 96 rats were divided in 4 groups: (1) control, (2) ELF-MF (50 Hz, 400 μ T, continuous 60 d), (3) AD, and (4) AD + (ELF-MF). Groups 2 and 4 were ELF-MF exposed for 60 days; groups 1 and 3 were not exposed. Obviously, in group 4, the 42 days lasting ip and d43-microinjection were performed in parallel to the ELF-MF exposures. ELF-MF effects on AD development was studied by applying the Morris water maze at

6 h, 7 d, 14 d, 28 d after termination of exposure. Molecular-biological analysis of the hippocampus was done in n=5/group at 6 h, 7 d, 14 d, 28 d post exposure. ELF-MF (50 Hz, 400 μ T, continuous 60 d) partially improved the spatial learning of AD rats. But the average escape latencies (AEL) in the MWM were similar between groups 2 (ELF-MF) and 4 (AD+ELF-MF). Proteomic analysis demonstrated the involvement of several proteins of the RKIP-mediated NF- κ B pathway signaling. Besides the weakness of a missing sham-exposed AD control, this paper also suffers from a confusing description of the results.

2.3.2. Oxidative stress

Budziosz et al. (2018) evaluated possible effects of a 28-day exposure (22 h/d) to an ELF-MF on the oxidative stress in specific brain structures in male 10-week-old Wistar rats. Ten rats were ELF-MF-exposed (50 Hz, 10 kV/m, magnetic induction of 4.3 pT), another ten sham-exposed. Following the 28-day exposure, the rats were fasted for 24 h and the brains were removed after euthanasia. Homogenates of the frontal cortex, hippocampus, brainstem, hypothalamus, striatum, and cerebellum were obtained. The following parameters of oxidative stress were evaluated in the homogenates: total oxidant status (TOS), malondialdehyde (MDA), superoxide dismutase (SOD) and its isoenzymes (copper-zinc dismutase (SOD-CuZn), manganese dismutase (SOD-Mn)), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), and total antioxidant capacity (TAC). Following the 28-day exposure, the mean TOS and MDA levels in the different brain structures were similar between the two groups. Except for the frontal cortex, CAT, GPx, and hippocampal GR were decreased, i.e., the applied ELF-MF had no effect on the non-enzymatic antioxidant system. Overall, the 28-day exposure did not affect oxidative stress in the evaluated brain structures.

Seif et al. (2018) exposed two groups (n=7/group) of male Wistar rats to 50 Hz 0.7 mT ELF-MF for 2 h/d during one month. A third group (n=7) of rats were sham-exposed. In addition and before ELF-MF exposure, rats of group no. 2 were intraperitoneally treated with *Myrtus communis* extract (0.5 mg/kg). Heart blood samples were obtained after termination of (sham) exposures. Hemoglobin (Hb), methemoglobin (metHb) and hemichrome levels, the absorption spectrum of Hb (200-700 nm), protein carbonyl (PCO) levels and FRAP (ferric reducing ability of plasma) were determined. ELF-MF led to decreased FRAP and accordingly increased plasma PCO, metHb and hemichrome levels. The pre-exposure injection of *M. communis* extract followed by 1-month ELF-MF exposure resulted in increased FRAP and decreased plasma PCO, metHb and hemichrome concentrations, compared to ELF-MF only. According to the authors a significant increase in Hb absorbance at 340, 420, 542, and 577 nm showed protective properties of *M. communis* extract against ELF-MF-induced oxidative stress in erythrocytes. Furthermore they concluded that changes in Hb conformation could be associated with the formation of oxygen free radicals due to exposure to the magnetic fields.

2.3.3. Cytokines and miRNA

Li et al. (2018a) tested whether ELF-MF exposure dysregulates serum levels of specific cytokines which the authors considered might be a prerequisite for establishing a relationship between the occurrence of malignancy and PFEMF exposure [PFEMF= power frequency electromagnetic field]. Groups of n=100 four-week old male BALB/c mice were exposed to 50 Hz ELF-MF of 0 (sham), 0.1, 0.5 and 2.5 mT for 90 days, 8 h/d. Blood/serum was collected at five different timepoints (0, 1, 10, 30, 90 days) of n=20 mice/group each timepoint, thereafter the animals were humanely killed. The serum samples were used for the Luminex assay of the following chemokines: EOTAXIN-1 (CCL11), GRO α (CXCL1), IP-10 (CXCL10), MCP-1 (CCL2), MCP-3 (CCL7), MIP-1 α (CCL3), MIP-1 β (CCL4), MIP-2 (CXCL2), and RANTES (CCL5). Between the control and ELF-MF-exposure groups body weight development did not differ. But circulating chemokines were exposure-affected. Monocyte chemoattractant protein (MCP)-3, macrophage inflammatory protein (MIP)-1 α , MIP-1 β and MIP-2 levels were similar during the 3-month exposure period. Other chemokines including IP-10, GRO α ,

RANTES, EOTAXIN-1 and MCP-1 exhibited significant changes upon treatment. Among the responsive chemokines, EOTAXIN-1 and MCP-1 were significantly increased by 0.5 mT at all four post-exposure timepoints (1, 10, 30, 90 days), whereas the 0.1 mT and 2.5 mT exposed mice did not show this increase. Consequently the authors discussed that the non-dose-dependent responses of chemokine is out of their expectation. This phenomenon may reflect that the body has an unknown mechanism to respond to different electromagnetic field strength which is worth to further explore. Considering this observation, their final conclusion on a “novel finding of the induction of EOTAXIN-1 and MCP-1 during this process supports that these two chemokines could be used as circulating indicators for PFEMF exposure and highlights the potential pro-inflammatory nature of PFEMF...” is hard to understand. Nevertheless, study design and study size should be a starting point for re-evaluation studies on the observed effects and the discussed potential link(s) of the above chemokines to cancer development including childhood leukemia.

Li et al. (2018b) obviously used the same serum samples of the above described mouse experiment. After the same exposures and durations, 10 serum samples from the same exposure-time points were combined, exosomes⁶ were isolated and microRNAs (miRNA) extracted. After small RNA sequencing and qRT-PCR (quantitative real-time PCR) validation, serum exosomal miRNA biomarkers were proposed for the detection of different ELF-MF exposures. The researchers demonstrated dose-different expression of exosomal miRNAs, e.g. miR-128-3p for 0.1 mT, miR133a-3p for 0.5 mT, miR-142a-5p for 2.5 mT, and for all tested ELF-MF exposures miR-218-5p and miR-199a-3p. Besides the potential of miRNAs to detect ELF-MF exposures and intensities, exosomal miRNA markers were predicted to be involved in critical pathophysiological processes of neural system and cancer- or other disease-related signalling pathways.

The exposure duration, i.e. five different timepoints (0, 1, 10, 30, 90 days) were not explicitly addressed. In addition, these first time-observations need to be confirmed by other research groups.

Mahdavinejad et al. (2018) tested the effects of four different ELF-MF (50 Hz, flux intensities 1, 100, 500 μ T and 2 mT) on serum levels of interleukin 17 (IL-17), transforming growth factor- β (TGF- β) as well as on expression levels of retinoid-related orphan receptor γ T (*ROR γ T*) and transcription factor forkhead box P3 (*Foxp3*) in spleen and thymus of rats. Four groups (n=16/gr) of 8-week-old male Wistar rats were exposed to the above ELF-MF for 2 months (2 h/d), a fifth group was sham-exposed. After a 1-month exposure, 5 mL blood of all animals was collected and serum separated for cytokine measurements. For stimulation of the immune system, all rats were immunized by human serum albumin (HSA, 100 mg/rat ip) on days 31, 44, and 58 of exposure to ELF-EMF. After termination of the of 2-month-exposure period, the rats were sacrificed under ether anesthesia. Total blood, spleen and thymus were collected. Body weight and spleen weight did not differ between the groups, whereas the thymus weight of 2 mT ELF-MF-exposed rats was significantly reduced. After 1 month (preimmunization phase), the serum levels of IL-17 and TGF- β were significantly decreased at 1 and 100 mT. After two months (postimmunization phase), IL-17 and TGF- β levels were similar between the groups. Compared to sham controls, the expression levels of *ROR γ T*, *Foxp3* genes in thymus, and *ROR γ T* in spleen were not significantly changed following exposure to different intensities of ELF-EMFs. But the normalized expression levels of *Foxp3* in the spleen were significantly downregulated at intensities of 1 and 100 μ T. Summarizing, low intensities of ELF-EMF (50 Hz, 1 and 100 μ T) reduced the serum levels of IL-17 and TGF- β and downregulated the expression of *Foxp3* in spleen. Since IL-17 and TGF- β were used as signature cytokines of Th17 and regulatory T (Treg) cells, the authors discuss that functions of both Th17 and induced Treg cells might be suppressed by ELF-EMF. But the higher intensities of 500 μ T and 2 mT did not show this effect.

Sobhanifard et al. (2019) obviously used blood, spleen and thymus samples from the above very same rats. They reported on interferon (IFN)- γ and interleukin (IL)-4 levels in blood serum on days 31 and 61, and on the expression of T-box transcription factor (T-bet) and GATA binding protein-3 (GATA-3) genes in the spleen and thymus on day 61, i.e. post immunization. Serum IFN- γ and IL-4 levels were significantly reduced at prestimulation (day 31) after 100 μ T ELF-MF only. Expression of T-bet

⁶ Exosomes are nanosized lipid vesicles (30 – 90 nm) released from cells. They are capable of transferring proteins, mRNA, and miRNA between cells and, therefore, represent a potential means of intercellular communication.

and GATA-3 mRNA was significantly decreased in the spleen in rats exposed to densities of 1 and 100 mT, whereas the expression after exposure to 500 μ T and 2 mT was not different to sham controls. Thymic T-bet or GATA-3 expression was similar in all (sham) exposure groups. Regarding timepoint and ELF-MF exposure level(s) the findings are consistent with Mahdavinejad et al. (2018). The modeling of splenic T-bet and GATA-3 mRNA expression and serum IFN- γ and IL-4 levels (during prestimulation phase) and various flux magnetic densities (1, 100, 500, and 2000 μ T) resulted in a parabolic curve. Taking their results together the authors concluded that some functions of both Th1 and Th2 cells were compromised due to ELF-MF exposures at lower densities, but the effect is transient and apparently related to the immune activation status of a given host.

Wyszkowska et al. (2018) evaluated pro-inflammatory cytokines (IL-1 β , IL-2, IL-6, IL-10) in rats following exposure to ELF-MF. Five groups of n= 6 male 3-month-old Wistar rats were used: (1) non-treated (NT), (2) single continuous exposure (50 Hz, 7 mT for 1x 24 h), (3) repeated exposure (50 Hz, 7 mT, 7x 1h/d, 7d), (4) sham 1x 24h, (5) sham 7x 1h. Following exposure, blood samples were taken via cardiac puncture. Hematology was done with 50 μ L whole blood. Then in blood plasma and using enzyme-linked immunosorbent assay (ELISA) the above interleukines were determined. The single 24 h exposure caused an increase in white blood cells, lymphocytes, hemoglobin, and hematocrit levels; also plasma IL-1 β , IL-2 and IL-6 were significantly increased compared to all other groups (NT, sham and 1x 7h). IL-10 was not different between all groups. Repetitive ELF-MF exposure (50 Hz, 7 mT, 7x 1h) did not change hematology and plasma cytokines. Concluding, the exposure duration (continuous vs. short (1h) repetition) is important for the immune response.

2.3.4. Physiology

Di et al. (2018) compared effects of 35 kV/m static electric field (SEF) (0 Hz) and 35 kV/m power frequency electric field (50 Hz, ELF-EF) on haematological parameters in mice. [The SEF part of the study is described in chapter 1.3.2.]. Groups of n=10 male 4-week old ICR mice were continuously exposed to ELF-EF for 7, 14, and 21 days, another n=10 mice/timepoint served as non-exposed controls. Following 7, 14 or 21 days of exposure to ELF-EF, blood was taken and the following parameters determined: White and red blood cell (WBC and RBC) count, hemoglobin (Hb) concentration, differential blood count (NE%, LYM%, MO%, EO%, BAS%). Compared to non-exposed control mice, after exposure to 35 kV/m ELF-EF the WBC significantly decreased after 1, 2 and 3 weeks. In ELF-EF blood a significant reduction in RBC count was shown after 7 days, and in Hb-concentration after 21 days, while for the other timepoints no differences were seen. Finally, the differential blood count was similar between the non-exposed and exposed mice.

The presented data do not justify for the authors conclusion "...in mice...exposure of 35 kV/m could cause a decline of immune function", while the different effects of SEF vs. ELF-EF on immune function was taken as "possibly caused by the difference of the degree of molecular polarization and ion migration in organism under exposure of two kinds of electric fields".

Hori et al. (2018) reported a further complex follow-up experiment to Hori et al. (2017) and Harakawa et al. (2017) (described in the 12th Council report (SSM, 2018)). Again, plasma glucocorticoid (GC) levels as an indicator for stress response were determined in 50 Hz and 60 Hz electric field (EF)-exposed male BALB/c mice. In addition, red and white blood cell counts (RBC, WBC), hemoglobin and hematocrit levels were analyzed. Groups of n=6-8 eight-week-old mice were exposed to 10 kV/m for 60 min, and tube-immobilized for 30 min between minute 30 to 60. Immediately after (EF)treatment, of all mice blood samples were collected.

Test 1 aimed to examine whether the frequency (50 or 60 Hz) of the electric field (EF, 10 kV/m) influenced the immobilization-induced ("stress") increase in GC plasma glucocorticoid (GC) levels, reported by the same group in 2015 and 2017.

Test 2 was designed to test a potential influence of illuminance on the effect of EF exposure with or without restraint stress.

Test 3 addressed the effect of partial or complete shielding on EF-exposed and/or restraint-stressed mice.

In all tests and compared to control group(s), plasma GC levels in restraint [Stress] mice were 2 to 4.5 times increased. GC was lower in the stress-EF co-treated groups compared to the immobilization-alone group, but there was no difference in GC levels and WBC count between the groups EF-alone (50 and 60 Hz) and controls, whereas RBC count, HGB and HCT levels were lower in the control than in the treated groups (Test 1). Overall, illuminance level was not correlated to plasma GC level and blood parameters (Test 2). Mice' shielding from the EF inhibited the EF effect which was negatively correlated to the area shielded (Test 3). The authors demonstrated that ELF-EF (50 and 60 Hz, 10 kV/m) suppresses changes of the endocrine system induced by acute immobilization stress. In addition, the suppressive effect of EF exposure depends on the body surface area exposed.

Martinez-Samano et al. (2018) evaluated the ELF-MF exposure (60 Hz, 2.4 mT), restraint stress (RS) or both (RS + ELF-MF) on lipid profile and lipid peroxidation in the brain of Wistar rats that were allocated into four groups (n=6/gr): (1) control, (2) RS, (3) ELF-MF, and (4) RS + ELF-MF. After 2 h/d and 21 days lasting treatment of rats, blood was obtained for quantitative plasma corticosterone concentration and their brains were dissected in cortex, cerebellum and subcortical structures for analysis of cholesterol, triacylglycerols, total free fatty acids, and thiobarbituric acid reactive substances (TBARS) analysis. Finally, fatty acid methyl esters (FAMES) were identified. Compared to controls, plasma corticosterone levels were increased in RS (2) and ELF-MF (3) exposed groups, being higher in the RS + ELF-MF group (4). ELF-MF exposure increased total lipids in cerebellum, and total cholesterol in cortex, but decreased polar lipids in cortex. In subcortex, non-esterified fatty acids were increased in the RS + ELF-MF group (4). In cerebellum, polyunsaturated fatty acids were decreased but increased in subcortex of ELF-MF exposed rats. TBARS concentration in lipids was increased in all treated groups (2-4) compared to the control group, especially in cortex and cerebellum. The authors concluded that chronic exposure to ELF-MF is similar to physiological stress, and induces changes on brain lipid profile.

2.3.5. Reproduction and development

Park et al. (2018) exposed 7-week old male Sprague-Dawley rats (n=12/gr) to 60 Hz MF of 2, 20, or 200 μ T for 24 h/day. A fourth group of 12 rats was exposed to sham conditions. Body weight in all four groups developed similar. After 20 weeks of exposure, specimens of epididymides and testes of each animal were investigated for potential effects on testicular function. Testis mass was similar in all groups. The exposure to 60 Hz MF of 2 μ T and 20 μ T had no effects on testicular function (apoptosis, diameter of seminiferous tubules, sperm count, motility and morphology). But the exposure to 200 μ T induced increases of the apoptotic cells in germ cells and decreases sperm numbers. Seminiferous tubule diameter or sperm motility and morphology were not affected. Finally, the authors of the Korean research group stated that compared to previous mice studies, rats are less sensitive than mice to exposure to 60 Hz MF.

Ruan et al. (2019) reported a series of studies on the effects of 50 Hz ELF, 30-500 μ T MF on fertility and development in rats and mice. In each study adult Sprague-Dawley rats and C57BL/6J mice were divided in 4 groups: sham, 30, 100, 500 μ T exposure groups. The exposure duration per day was 20 h. Rats: 15 males and 15 females per group were exposed for 24 weeks. None of the ELF-MF exposures affected body weight or paired ovary, testis and epididymis weight and sperm count. In addition, no significant differences in plasma sex hormones (estradiol and progesterone in females, testosterone in males) were found.

Mice: (A) Per group 8 female and 8 male C57BL/6J mice (8-wk old) were exposed for 12 weeks. Following ELF-MF exposure, again no differences in plasma estradiol and progesterone (female mice) and in testosterone (male mice) were seen. (B) For the determination of the pregnancy rate each male mouse of the following groups was caged with two females: (1) sham females (n=10) x sham males (n=5), (2) sham females (n=10) x ELF-MF males (n=5), (3) ELF-MF females (n=10) x sham males (n=5). Before mating mice of groups (2) and (3) were exposed to ELF-MF (30, 100, 500 μ T) for 8 weeks. These mating/pregnancy rate studies were repeated three times independently, i.e. in total 315

mice were used. Eight days after successful mating, pregnancy rates and number of implanted embryos were not significantly different between the four sham and exposure groups.

(C) Again, 120 female and 60 male mice were mated 2:1. Plug positive females were divided in four groups (n=30/group) and exposed to 0 (sham), 30, 100, and 500 μ T till gestation day 18. There were no differences in numbers of viable and dead fetuses between the groups. No resorptions and no external abnormalities were reported.

(D) Sixty female mice were mated with 30 males. Neonatal mice were randomly divided into four groups (n=30/gr) and together with their mother exposed to 0 (sham), 30, 100, and 500 μ T for 3 weeks. Mean body weight and pups' development (time point of eye opening and of tooth eruption) did not differ between the groups. Overall, the authors did not find significant differences between the sham and exposed groups for all parameters tested with respect to fertility and reproductive development.

2.3.6. Studies in non-mammalians

Shepherd et al. (2018) tested how acute exposure to 50 Hz ELF-MF levels similar to ground levels of overhead powerlines (20-100 μ T) or found within 1 m distance of the conductors of power lines (1 mT – 7 mT) may impair the cognitive and motor abilities of honey bees.

(1) For the analysis of effects on associative learning, in total 438 bees (n \geq 100/gr) were exposed to 3 different ELF-MF levels (20, 100, 1000 μ T) or sham-treated for 1 min immediately following conditioning trials. According to the authors this simulated a realistic scenario of exposure of flying bees in the field crossing an EMF boundary of a powerline immediately after location/returning to a food source. Using the proboscis extension response (PER), ELF-MF exposure reduced significantly and MF intensity-dependently the final learning level (after 5 trials) of 73% in sham compared to 63% in 20 μ T, 42% in 100 μ T and 36% in 1 mT exposed bees.

(2) Applying the Tethered Flight test, 120 bees (n = 30/gr) were exposed to 0.1, 1, and 7 mT. By use of high-speed video the wingbeat frequencies of bees 0.5 s before ELF-MF exposure and 2.5 s after ELF-MF exposure were determined. Compared to sham all ELF-MF exposures caused MF intensity-dependently an increase in wingbeat frequency.

(3) Foraging experiments were done with bees from 6 nucleus hives. Following 15 min recording of baseline feeding and flight levels without ELF-MF exposure, another 15 min recordings of flight and feeding levels during control or 100 μ T ELF-MF exposures were made. In total, 3 699 feeding events were recorded. Exposure to 0.1 mT ELF-MF significantly reduced the percentage of successful outgoing flying passes from the hive to the feeder. Summarizing, ELF-MF exposure reduced learning, the success of foraging flights towards food sources, and feeding. In line with the authors, the tested ELF-MF may be an environmental stressor for honey bees, having impact on their cognitive and motor abilities.

Kim et al. (2018) investigated the influence of pulsed electromagnetic fields (PEMFs) on pigmentation in Zebrafish (*Danio rerio*). Fertilized embryos were individually placed in 96-well plate filled with 100 μ L sea salt-containing water. A Helmholtz coil-based exposure unit was used. For 5 or 15 days the Zebrafish embryos were exposed to PEMFs (60 Hz, “the stimulus wave was in a pulse form” and at “intensities of 2, 4, and 20 G”, i.e. 0.2, 0.4, and 2 mT). A separate incubator served for the sham exposure. Melanin, melanogenesis-related genes, pigmentation, and pigmentation-related proteins were evaluated at 5 or 15 day post-fertilization (dpf), equal to exposure duration. PEMF increased the melanin content at 5 dpf (1.22-, 1.32-, 1.16-fold after 0.2, 0.4, 2 mT exposure compared to control, n=80). Exposure to 0.04 and 2 mT resulted 5 dpf in a up to 2-fold increase of key melanogenesis-related genes ([*dct*, *tyrp1*, *mitfa*, *mcr1r*], n=30). After 0.4 mT exposure and 15 dpf the expression of all pigmentation-related proteins (especially TRP1, MITF, DCT) were significantly increased. Correspondingly, a significantly increased pigmentation after 0.4 mT exposure was demonstrated 15 dpf.

Unfortunately, protein and pigmentation data of the other groups/exposure levels were not shown; i.e., the method of data presentation is suggestive of an exposure optimum at 0.4 mT.

The authors conclude that PEMFs promote pigmentation by inducing MITF and DCT, which are

mediated through a reduction of ERK phosphorylation and an upregulation of p38 phosphorylation. Without any related substantiation they stated these results suggesting that PEMFs, at an optimal intensity and frequency, are a useful tool for treating gray hair with reduced melanin synthesis in the hair shaft, or hypopigmentation-related skin disorders such as vitiligo.

Sun et al. (2018c) evaluated some physiological responses of *Caenorhabditis elegans* (*C. elegans*) to ELF-MF (50 Hz, 3 mT) and sham exposure. *C. elegans* worms were exposed from egg to fourth larval (L4) stage. Approximately 15 000 worms (in three plates) were exposed until reaching the L4 stage, usually after \approx 48 h. The same was done for the (non-exposed) sham-group. After (sham) exposure L4-stage worms were washed in buffer and harvested by centrifugation. Tricarboxylic acid (TCA) cycle enzymes were determined by qRT-PCR and western blot analysis, two lipid metabolites [arachidonic acid (ArA), prostaglandin E₂ (PGE₂)] by GC-MS (gas chromatography-mass spectrometry), reactive oxygen species (ROS) level by dichlorofluorescein staining and finally, the worm antioxidant system was tested by superoxide dismutase (SOD) and catalase (CAT) activities, and the total antioxidant capacity (T-AOC). ELF-MF exposure resulted in decreased expression of the (TCA cycle enzyme) fumarase. ArA and PGE₂ concentrations were elevated, the expression of prostaglandin E₂ synthase increased. Corresponding to an increased ROS level, a significant depression of T-AOC was found in response to ELF-EMF. The authors concluded that exposure to 50 Hz, 3 mT ELF-EMF in *C. elegans* can elicit disruptions of the TCA cycle metabolism and PGE₂ formation, coupling ELF-EMF-induced oxidative stress responses.

2.3.7. Summary and conclusions on ELF animal studies

Similar to the previous Council reports, studies used exposure levels mostly in the 1 mT range and below at 50 or 60 Hz. The different studies described various and partly contradictory effects of ELF-MF exposure in rodents, but did not provide insight on potential ELF-MF mechanism(s).

The environmental study on honey bees showed that ELF-MF may be an environmental stressor for flying insects, having impact on their cognitive and motor abilities. However, this only underlines the absence of knowledge on biological-relevant mechanisms of ELF-MF. The Zebrafish study addressing pigmentation may be of minor relevance; but Zebrafish studies are a useful tool addressing fertility, cardiovascular system etc. Analogously also round worm (*C. elegans*) is used in basic research. Finally, no study directly addressed childhood leukemia. But the increasing number of studies using the endpoint ‘Cytokines’ may indirectly address leukemia in future, e.g., the study of Li et al. (2018a).

Table 2.3.1. Animal studies on exposure to ELF magnetic fields

Endpoint	Reference	Exposure ELF - MF	Exposure Duration and Species	Effect
Rodent studies				
Brain & behaviour	Zuo et al. (2018)	50 Hz, 400 μ T	24h/d, 60d Rat	Improved learning in AD rats, key role of NF- κ B pathway.
Oxidative stress	Budziosz et al. (2018)	50 Hz, 10 kV/m, 4.3 pT magnetic induction	22h/d, 28d Rat	Oxidative stress was not affected in different brain structures.
	Seif et al. (2018)	50 Hz 0.7 mT /+ <i>M. communis</i> /	2h/d, 1 mo. Rat	FRAP \downarrow PCO \uparrow methHb \uparrow hemichrome \uparrow /protective to oxidative stress of ELF-MF/
Cytokines	Li et al. (2018a)	50 Hz 0.1, 0.5, 2.5 mT	0, 1, 10, 30, 90d; 8h/d Mouse	EOTAXIN-1 & MCP-1 induced by 0.5 mT. No dose-dependent response!
	Li et al. (2018b)	50 Hz 0.1, 0.5, 2.5 mT	0, 1, 10, 30, 90d; 8h/d Mouse	Serum exosomal miRNAs aberrantly expressed compared to different MF strengths
	Mahdavinejad et al.	50 Hz	2 mo., 2h/d	1&100 μ T: IL-17 \downarrow &

	(2018)	1,100, 500, 2000 μ T + HSA immunization (100 μ g/rat on days 31, 44, 55)	Rat	TGF- β \downarrow in serum, Foxp3 downregulated in spleen. 2mT: thymus weight \downarrow
	Sobhanifard et al. (2019)	50 Hz 1,100, 500, 2000 μ T + HSA immunization (100 μ g/rat on days 31, 44, 55)	2 mo., 2h/d Rat	100 μ T: IFN- γ \downarrow & IL-4 \downarrow in serum. 1&100 μ T: T-bet & GATA-3 downregulated in spleen.
	Wyszkowska et al. (2018)	50 Hz, 7 mT	1x 24h once 7x 1h, 7d Rat	Only after 1x 24h blood parameters (WBC, LYMPH, HB, HCT) and plasma cytokines (IL-1 β , IL-2, IL-6) increased.
Physiology	Di et al. (2018)	35 kV/m SEF	7, 14, 21d 24h/d Mouse	WBC decreased (7, 14, 21d), RBC (7d), Hb (21d). DIFF not affected.
	Hori et al. (2018)	50 Hz, 60 Hz 10 kV/m + 30 min restraint stress	1 h Mouse	EF (10kV/m) partly suppressed stress- induced GC increase. Shielding inhibited this EF effect.
	Martinez-Samano et al. (2018)	50 Hz 2.4 mT + restraint stress	2h/d, 21d Rat	ELF-MF similar to restraint stress (increase of plasma CCN, total lipids in cerebellum, cholester- ol in cortex, TBARS in lipids etc.).
Reproduction & development	Park et al. (2018)	60 Hz, 2, 20, 200 μ T	24h/d, 20wk Rat	No effect on bw, testis mass, sperm motility & morphology. Apopto- sis \uparrow & sperm num- bers \downarrow at 200 μ T only.
	Ruan et al. (2018)	50 Hz, 30, 100, 500 μ T	24wk (rat) 18d – 12wk (mice) Rat Mouse	No differences in bw, plasma sex hormone levels, pregnancy rates, growth and development of neonatal mice.
Studies in non-mammalians				
Cognitive & motor abilities	Shepherd et al. (2018)	50 Hz, 20, 100, 1000 μ T; 0.1, 1, 7 mT	Acute (1 min) Honey bee	MF-dependent decrease in learning; increase in wingbeat frequency. Reduction in foraging flights (100 μ T).
Pigmentation	Kim et al. (2018)	60 Hz PEMF 0.2, 0.4, 2 mT	5dpf, 15 dpf Zebrafish	Increase of melanin content, melanogene- sis-related genes, pigmentation-related proteins and pigmen- tation.
Oxidative stress & tricarboxylic acid cycle	Sun et al. (2018c)	50 Hz 3 mT	\approx 48h C. elegans	ROS \uparrow , T-AOC \downarrow , Fumarase \downarrow , ArA \downarrow & PGE ₂ \downarrow

Abbreviations: \uparrow =increase(d); \downarrow =decrease(d); ArA: arachidonic acid; bw: body weight; CCN: corticosterone; DIFF: differential blood count; dpf: days post fertilization; EF: electric field; ELF-MF: extremely low frequency magnetic field; EOTAXIN: subfamily of eosinophil chemotactic proteins; Foxp3: transcription factor forkhead box P3; FRAP: ferric reducing ability of plasma; GATA-3: GATA binding protein-3; GC: glucocorticoid(s); Hb: haemoglobin concentration; HCT: haematocrit; IFN- γ : interferon- γ ; IL: interleukin; LYMPH: lymphocytes; MCP: monocyte chemoattractant protein; methHb: methaemoglobin; miRNA: microRNA; NF- κ B: nuclear factor k-light-chain-enhancer of activated B cells; PCO: protein carbonyl; PGE₂: prostaglandin E₂; RBC: red blood cell count; ROS: reactive oxygen species; SMF: static magnetic field; STZ: streptozotocin; T-AOC: total antioxidant capacity; T-bet: T-box transcription factor; TGF- β : transforming growth factor- β ; WBC: white blood cell count.

2.4. Cell studies

Among the 11 papers found in the period of interest, three have not been included in the analysis due to scanty quality of the research. The eight studies considered addressed the effect of exposure on genotoxicity, cell viability and proliferation, oxidative stress, apoptosis and epigenetic effects.

2.4.1. Genotoxic effects

Ross et al. (2018) investigated the induction of genotoxic and cytotoxic effects induced in human mesenchymal stromal cell (hMSC) line by intermittent exposures (20 min/day, 3 days/week for 2 weeks) to 5 Hz, 0.4mT. In three independent experiments, karyotype analysis did not reveal differences between exposed and sham-exposed cultures. Cell viability and proliferation were also unaffected.

Ataxia telangiectasia mutated (ATM) gene plays a central role in DNA double-strand break (DSB) repair. Sun et al. (2018a) employed mouse embryonic fibroblasts (MEFs) both wild-type (*Atm*^{+/+}) and *Atm*-deficient (*Atm*^{-/-}) to investigate the effects of 50 Hz MF exposure, 2 mT field intensity, on DNA damage, cell viability and cell cycle progression. For DNA damage and cell cycle progression the exposure duration was 15 min, 1 h or 24 h, while to evaluate cell viability, cultures were exposed or sham-exposed for 1 h or 24 h. Treatments with 0.1 mM 4-nitroquinoline 1-oxide for 1 h served as positive control and worked properly. The results of three independent double blinded experiments indicated absence of DNA damage, evaluated as foci formation and DNA migration (comet assay), as well as alterations in viability and cell cycle progression in both cell types and for all the experimental conditions investigated.

2.4.2. Oxidative stress

In a previous paper, the research group of Sun demonstrated the ability of 50 Hz, 0.4 mT MF exposure, to induce epidermal growth factor receptor (EGFR) clustering and phosphorylation on cell membranes (Ke et al., 2008). In order to elucidate the mechanisms behind such an effect, the authors evaluated the possible role of reactive oxygen species (ROS) formation by exposing human amnion epithelial (FL) cells (Sun et al., 2018b). Exposures of 5, 15 and 30 min resulted in enhanced ROS levels compared to sham controls ($p < 0.05$). Such an enhancement was negated in presence of N-acetyl-L-cysteine (NAC) or pyrrolidine dithiocarbamate (PDTC), two ROS scavengers ($p < 0.05$; three independent double blinded experiments). When exposed cultures were compared with the sham group, a significant increase in the ratio of EGFR clustered cells and in the acid sphingomyelinase activity, an enzyme involved in EGFR clustering, was detected ($p < 0.05$). Such effects were inhibited by NAC or PDTC treatment, suggesting the involvement of ROS in MF-induced EGFR clustering.

In a study conducted by Consales et al. (2018) the response to ELF-MF (50 Hz, 1 mT) was characterized in an *in vitro* model of familial Amyotrophic Lateral Sclerosis (fALS), carrying two mutant variants of the superoxide dismutase 1 (SOD1) gene. The experiments were carried out on human SH-SY5Y neuroblastoma cells transfected with human wild-type SOD1 (SOD1^{WT}) or mutant SOD1 (SOD1^{G93A} or SOD1^{H46R}). Cell cultures were exposed for 24 h and up to 72 h on the basis of the biological endpoint analysed. The results of three to five independent experiments indicated a significant deregulation in the expression of iron-related genes IRP1, MFRN1 and TfR1 was recorded in the SOD1^{G93A} clone and associated with a slight ($P = 0.05$) difference in the total iron content. This result was not associated to ROS formation and H₂O₂ levels; in addition, proliferation, viability, and apoptosis also resulted unaffected. The authors concluded that 50-Hz MF affects iron homeostasis in the *in vitro* SOD1^{G93A} ALS model.

2.4.3. Other cellular endpoints

Rescigno et al. (2018) exposed human osteosarcoma SaOS-2 cells and human breast cancer cell lines MCF-7 and SK-BR-3 to a 75 Hz, 1.5 mT ELF-EMF for 1h to evaluate cell viability. In three independent experiments no differences were detected on viability with respect to sham exposed cultures in the cell types tested. In addition, to address the ability of ELF exposure to promote osteogenesis, alkaline phosphatase enzymatic activity and protein expression were measured in SaOS-2 cells at different times after exposure (0, 4 and 24 h). Treatment significantly up-regulated protein activity and expression already immediately post exposure ($p < 0.01$). Such an increase was also detected 4 and 24 h post-exposure. mRNA levels were not affected.

Yuan et al. (2018) used Human neuroblastoma G401, mouse neuroblastoma N2a and Human neuroblastoma CHLA255 cell lines to evaluate the number of live cells after exposure to a 50 Hz MF, 5.1 mT, given 0.5, 1 or 2 h/day for 1, 2 and 3 days. In three independent experiments a reduced cell number was detected after 2 and 3 days exposure in all the cell types investigated ($p < 0.05$). In addition, the authors detected a reduced proliferation rate and an increased number of apoptotic cells after exposure of 2 h/day at 2 and 3 days exposure in G401 cells ($p < 0.05$). They also demonstrated a potentiation of cisplatin (DPP)-induced cell number reduction when the three cell types were subjected to combined treatments (MF+DDP).

In a study carried out by Wang et al. (2018b) the effect of 2, 4 and 6 h exposure to 50 and 120 Hz, 6 mT field intensity, was evaluated on differentiated and undifferentiated PC12 cells, a cell line from rat pheochromocytoma. The results of three independent experiments indicated no effect on ATP level in both cell types after exposure to 50 Hz. At 120 Hz a slight but statistically significant increase in undifferentiated cells was found at 4 and 6 h exposure ($p < 0.05$). To evaluate if such an effect is cell type-dependent, nine more cell lines, such as five human cancer cell lines (HeLa, HCT116, MCF7, A549, and GIST-T1), a rat cancer cell line (C6), and three non-cancer cell lines (RPE1, CHO, and 293T), were employed to test the ATP level after 2, 4 and 6 h exposure. In a few sporadic cases a statistically significant decrease or increase ($p < 0.05$) with respect to sham-exposed controls was recorded only in some but not in all the cells tested at some but not all exposure durations. In addition, the levels of mitochondrial membrane potential (MMP) and of ROS were measured in HCT116, 293T, GIST-T1 and RPE1 cells following 2 h exposure to 50 Hz. No effects were detected in MMP while ROS levels were increased in HCT 116 cells and decreased in RPE1 cells ($p < 0.05$).

Although such results have been obtained on a large number of cell types, they do not provide a clear evidence for an effect on a specific cell type. In this paper, the effect of exposure to SMF was also investigated and the results are reported in section 1.4.

Benassi et al. (2019) exposed SH-SY5Y cell line to 50 Hz, 1 mT, for 24 up to 72 h, to investigate the global DNA methylation. Both proliferating and differentiated SH-SY5Y cells were used. In addition, since epigenetics plays a key role in the neurodegenerative process, the response of ELF exposure was also considered in combination with 1-methyl-4-phenylpyridinium (MPP^+), a neurotoxin mimicking the Parkinson's Disease (PD) phenotype.

It is known that DNA repetitive sequences are heavily methylated, and it is estimated that more than a third of DNA methylation occurs in the repetitive sequences. To address the global DNA methylation, the authors assessed the methylation of three sequences: ALU, LINE-1 and SAT- α . When exposed and sham-exposed samples were compared, absence of effects on DNA methylation was detected in both proliferating and differentiated cells, either under basal and under PD mimicking conditions (three independent experiments).

2.4.4. Summary and conclusions for cell studies

The in vitro studies on ELF-MF do not indicate induction of genotoxic and epigenetic effects, while it seems that oxidative damage is slightly induced. Concerning the other endpoints considered, results are not univocal, with increase, decrease or no differences when exposed samples were compared to sham controls.

Table 2.4.1. Cell studies on exposure to ELF magnetic fields

Cell type	Endpoint	Exposure conditions	Effect	References
Human mesenchymal stromal cell line	Genotoxicity, viability and cell proliferation	5 Hz, 0.4 mT 20 min/day, 3 days/week for 2 weeks	No effects.	Ross et al (2018)
Mouse embryonic fibroblasts wild-type and Atm-deficient	DNA migration, viability and cell cycle progression	50 Hz, 2 mT 15 min, 1 h, 24 h	No effects.	Sun et al (2018a)
Human amnion epithelial cells	ROS formation, EGFR clustering	50 Hz, 0.4 mT 5, 15, 30 min	ROS increase, negated by NAC or PDTC. Increase in the ratio of EGFR clustered cells and in the acid sphingomyelinase activity, inhibited by NAC or PDTC.	Sun et al (2018b)
Human neuroblastoma (SH-SY5Y) cells transfected with human wild-type or mutant SOD 1 gene	Proliferation, viability, apoptosis, ROS formation, H ₂ O ₂ level, gene expression of iron-related genes	50 Hz, 1 mT 24 up to 72 h	Deregulation of IRP1, MFRN1 and TfR1 gene in mutant cells. No effects on the other endpoints	Consales et al (2018)
Human osteosarcoma SaOS-2 cells and human breast cancer (MCF7 and SK-BR-3) cell lines	Cell viability, enzyme activity, protein expression	75 Hz, 1.5 mT 1 h	No effects on viability. Up-regulation of alkaline phosphatase activity and expression	Rescigno et al (2018)
Human neuroblastoma G401; mouse neuroblastoma N2a; Human neuroblastoma CHLA255	Cell viability and proliferation, apoptosis	50 Hz, 5.1 mT 0.5, 1, 2 h/day for 1, 2 and 3 days Co-exposures with DPP	Reduced cell number and proliferation and increased number of apoptotic cells after 2 and 3 days exposure in all cell types. Potentiation of DPP-induced cell number reduction.	Yuan et al (2018)
Differentiated and undifferentiated rat pheochromocytoma (PC12) cells; human and rat cancer and non-cancer cell lines	ATP level, MMP, ROS formation	2, 4 and 6 h exposure to 50 and 120 Hz, 6 mT	No effect on ATP level in differentiated PC12 cells at 50 Hz; increase in undifferentiated cells at 120 Hz at 4 and 6 h. Sporadic increase or decrease in some but not in all the cells tested at some but not all the exposure durations. No effect on MMP; increased or decreased ROS levels in some cell types.	Wang et al. (2018b)
Human neuroblastoma (SH-SY5Y) cells	global DNA methylation, epigenetic effects,	50 Hz, 1 mT 24 up to 72 h Co-exposures with MPP+	No effects.	Benassi et al. (2019)

Abbreviations: ATP: adenosine triphosphate; DPP: Cisplatin; EGFR: epidermal growth factor receptor; MMP: mitochondrial membrane potential; MPP+: 1-methyl-4-phenylpyridinium; NAC: N-acetyl-L-cysteine; PDTC: pyrrolidine dithiocarbamate; ROS: Reactive oxygen species; SOD: superoxide dismutase.

3. Intermediate frequency (IF) fields

Last year's report observed that despite increasing use of intermediate frequency magnetic field (IF-MF) emitting sources (e.g. induction cooking, anti-theft devices), scientific evaluation of potential health risks was scarce – in fact, in the last report, no cell, three animal, no human and one epidemiological studies were identified. It was also noted that exposure assessment, especially of induced internal (electric) fields, remained challenging.

3.1. Epidemiological studies

Occupational exposure to IF-EMF and associations with brain tumours was investigated within the INTEROCC study (Comprised of the INTERPHONE centres in Australia, Canada, France, Germany, Israel, New Zealand and the UK) (Vila et al., 2018). In the years 2000-2004, as part of the INTERPHONE questionnaire, full lifetime job exposure histories of all jobs held for ≥ 6 months detailing proximity to specific sources of IF and RF EMF (0 Hz-300 GHz) were established for cases of glioma and meningioma as well as 1-2 matched controls per case. (The RF part of the study is summarized in the RF-chapter.) Average occupational exposure to H-fields from IF (3 kHz-10 MHz) devices were established by means of a source-exposure matrix (SEM) (Vila et al., 2017). Exposures from sources with different frequencies were combined using the ICNIRP reference levels as weights (ICNIRP 1998). Conditional logistical regression stratified by age, gender and country and adjusted for education was used to calculate OR associated with lifetime cumulative exposure with 1, 5 and 10 years lag time and with exposure in the time windows 1-4 and 5-9 years before diagnosis. Frequency-weighted exposures were dichotomized according to the median among occupationally exposed controls. The participation rates among glioma and meningioma cases were 65% and 78% respectively. Among controls it was 53 %, and the resulting study population comprised of 1 943 glioma and 1 862 meningioma cases and 5 387 controls. Only 1% of the participants had occupational exposure to IF magnetic fields, with the most common source being induction heater/furnaces for metals and the highest field strengths arising from electronic article surveillance. Among people with lifetime exposure ≥ 0.29 ICNIRP ratio-years, eight cases of glioma and seven cases of meningioma gave rise to an OR of 0.96 (95% CI: 0.42-2.21) and 1.43 (95% CI: 0.58-3.52) when compared to people never occupationally exposed to IF. When analysing other exposure windows the number of cases was even lower, yielding very imprecise OR estimates not significantly deviating from unity. The authors conclude that the results did not support an association between occupational IF magnetic fields and brain tumours.

The study benefits from a SEM that allows for greater inter-person variability in exposures than job-title based exposure matrices. However, as pointed out by the authors, the very low number of exposed participants, the reliance on ICNIRP regulatory limits rather than more biophysically founded ones, as well as the low participation rates are major limitations of the study.

3.1.1. Conclusions on IF epidemiological studies

Few studies have assessed potential health risks from the exposure to intermediate frequency fields. A re-evaluation of occupational IF-MF exposure in the INTERPHONE study did not provide evidence of an increased risk of glioma or meningioma among exposed workers.

3.2. Human studies

No human studies found for the reporting period.

3.3. Animal studies

Within the European GERoNiMo project a further animal study was published by the Finnish research group at UEF, Kuopio. Compare 13th Council report (SSM, 2019).

3.3.1. Genotoxicity

Herrala et al. (2018) studied possible genotoxic effects of IF-MF *in vitro* and *in vivo*. The *in vivo* experiment is described in the following. [The rat astrocytes vs. mouse blood cells are described in chapter 3.4.1]. Two groups of 10 two-months-old male C57BL/J mice each were exposed for 5 weeks (24 h/d) to 7.5 kHz MF at 12 μ T or 120 μ T. Further 10 male mice were sham-exposed. Following a 5-week exposure the animals were humanely killed and blood samples prepared for Comet assay and micronucleus (MN) assay. In blood samples of mice exposed to both, 12 μ T and 120 μ T MF, Comet assay showed decreased DNA damage compared to sham. Compared to sham, the MN level in peripheral blood erythrocytes (MnPCE) was lower in the 120 μ T group, but not statistically significant. Statistical overall differences between the 3 groups for MnPCEs were seen, with increased MnPCE level following 12 μ T exposure. The PCE/NCE ratio did not differ between the groups; i.e. the bone marrow was not affected by the IF-MF exposure. In conclusion, the *in vivo* results do not support genotoxicity of IF-MF.

3.3.2. Summary and conclusions on IF animal studies

In the 7.5 kHz range, in total four mouse studies (from the same Finnish research group, compare 13th Council report (SSM, 2019)) did not result in adverse effects on genotoxicity, fertility, reproduction, learning and behaviour. Again, it should be noted that the upper magnetic field strength (120 μ T) is about twice of nowadays cashiers' work place-exposures.

Table 3.3.1. Animal studies on exposure to intermediate frequency fields

Endpoint in rodents	Reference	Exposure IF - MF	Duration	Effect
Genotoxicity	Herrala et al. (2018)	7.5kHz, 12,120 μ T	24h/d, 5wk Mouse	No genotoxicity (Comet assay, MN test)

Abbreviations: IF: intermediate frequency, MF: magnetic field; MN: micronucleus.

3.4. Cell studies

3.4.1 Genotoxicity

Only one study was published in the period of interest, also including data on *in vivo* experiments.

Herrala and co-workers (Herrala et al., 2018) used rat primary astrocytes to test the genotoxic effects of IF, given alone and in combination with two well-known genotoxic agents. In particular, they employed menadione (MD, an agent that induces mitochondrial superoxide production and DNA damage, acting via ROS formation) and methyl methanesulfonate (MMS, an alkylating agent) at different concentrations.

Cell cultures were exposed for 24 h to a 7.5 kHz MF at a magnetic flux density of 30 or 300 μT . Co-exposures were investigated by testing different concentrations of chemicals (15 and 20 μM MD; 15 and 49 $\mu\text{g/ml}$ MMS). DNA damage and DNA repair were measured using the alkaline Comet assay and formation of micronuclei, assessed using flow cytometry. The results of three to four independent experiments did not support genotoxicity of IF exposure at both the field intensities tested. In experiments with co-exposure to MD, 15 μM induced no statistically significant differences in terms of DNA migration with respect to cultures exposed to MD alone. When a concentration of MD 20 μM was tested, measurements performed immediately after exposure showed a statistically significant decreased DNA migration in cells exposed at 30 μT ($p < 0.001$) and an increased damage at 300 μT ($p=0.001$), in comparison to the cultures exposed only to MD. In both cases a recovery over time was measured. MN frequency was not affected in all the co-exposure protocols with MD. MMS at 40 $\mu\text{g/ml}$ given in combination with 30 μT or 300 μT induced a decrease in DNA migration ($p<0.001$) for all the time-points investigated. Lower MMS concentration (15 $\mu\text{g/ml}$) resulted in an increased DNA migration at 30 μT but no effects were recorded at 300 μT . Both concentrations of MMS induced a statistically significant decrease of MN when co-exposures were carried out at 300 μT ($p<0.05$), while at 30 μT no combined effects were recorded. [In this study, MMS and MD treatments also served as positive controls for micronuclei and comets, respectively].

3.4.2 Summary and conclusions on cell studies

Only one study has been identified on the effect of IF on cell cultures, in the framework of the GERoNIMO project. As for the few studies published in the previous years, no effects of IF alone have been detected, but co-exposures evidenced cooperative effects that were dependent on the experimental conditions applied.

4. Radiofrequency (RF) fields

4.1. Epidemiological studies

Last year's report concluded that the possible association between mobile phone use and brain tumours was mainly addressed with time trends studies, pointing towards no association. It was also concluded that studies reporting decreased semen quality of mobile phone users generally lacked reliable RF-EMF exposure assessment of the testicles and that the studies were thus not able to solve whether observed associations are due to EMF exposure or other factors related to mobile phone use as such, for example lack of physical activity or stress level.

Similar issues were observed for various observed associations between behaviour and health-related quality of life in children and adolescents. Most of the studies observed associations but the underlying causal pattern was difficult to elucidate. In general, study quality was observed to be quite heterogeneous. Many low-quality studies appeared that did not fulfil basic quality criteria and were thus excluded from the review. On the other hand, some new approaches are promising to obtain new insights into potential health effects from radiofrequency field exposure.

4.1.1. Adult cancer

In the INTEROCC study (part of INTERPHONE) it was investigated if brain tumour risk was associated with occupational RF-EMF or IF-EMF exposure (Vila et al., 2018). The basic details of the study are given above under IF [3.1]. Ten percent of participants reported ever having had occupational exposure to RF-EMF sources; exposure was assigned to cases and controls using a Source Exposure Matrix (SEM). The most common occupational source of RF fields was occupational use of walkie-talkie (n=411). RF sealers/welders for plastic and rubber caused the highest E-fields (459 V/m). The highest H fields (0.91 A/m) came from dielectric heaters for plastic and rubber. ICNIRP reference levels were used as weights when combining different frequency sources and cumulative exposure indices were calculated as squared H- and E-fields to correlate with SAR values. An unweighted index reflecting internal magnetic field was also created. In general, ORs associated with occupational exposure were below unity when compared to people never occupationally exposed to RF-EMF. For the exposure period 1-4 years before diagnosis, elevated ORs for both glioma and meningioma were consistently seen in the highest decile of exposure. The number of cases in these segments were however small and confidence intervals were wide and always included one. The highest OR of 1.62 (95% CI 0.86-3.01) was observed for 19 glioma cases with cumulative RF H-field exposure of > 0.13 A/m-years. Overall, the study did not find evidence associating RF-EMF and brain tumours. However, the authors call for further investigations of the elevated point estimates among those with high exposures in recent years.

The limitations of the study are listed under the IF-part and relate to low participation, few high exposed cases and remaining exposure misclassification.

Using information from the INTERPHONE study conducted in Denmark, Finland and Sweden, Olsson et al. (2019) investigated if use of mobile phones prior to diagnosis influenced survival in glioma patients. A cohort was formed from the 876 glioma cases aged 20-69 and diagnosed in the years 2000-2002. Cohort members were followed up from diagnosis/interview until death, emigration of the country, to the date of the last follow-up, or completion of 10 years after diagnosis. Such information was obtained until 2014 (Denmark and Finland) or 2016 (Sweden). The initial response rate among INTERPHONE cases recruited between 2000 and 2002 was 71% in Denmark, 74% in Sweden and 91% in Finland. In this study, additionally 70 cases were excluded because of inconsistencies regarding diagnosis and follow-up dates. Mobile phone usage before diagnosis was assessed from the INTERPHONE questionnaire around time of diagnosis. Over 2 858 years of follow-up, 625 deaths were observed with median survival times of 10.4 months for glioblastoma patients (n=384), 19.8 months for other high-grade tumours (n=182) and >120 months for low-grade glioma

(deaths=236). For all three countries combined comparing regular mobile phone users with not regular users (never use and never ≥ 1 call per week for more than 6 months) resulted in a HR of 0.77 (95% CI: 0.61-0.98), 0.77 (0.63-0.94) and 0.68 (0.43-1.08) for glioma, all high grade and low-grade glioma respectively. The Cox regression models accounted for sex, age at diagnosis, treatment, anatomical location, marital status and country. The overall picture of no elevated HR among mobile users persisted in a range of sensitivity analyses investigating: years since first use, cumulative call time, usage in last 12 months prior to diagnosis, as well as education and comorbidity. The authors conclude that they saw no evidence of reduced survival among glioma patients according to mobile phone use before diagnosis and that the suggestion of longer survival among mobile phone users could result from residual confounding or prodromal symptoms among patients influencing the likelihood of taking up a new technology and becoming a regular mobile phone user (reverse causality). While benefiting from prospective exposure assessment and covering more than one country, the relatively small sample is a limitation. In addition, mobile phone use at baseline may not be representative for the follow-up and thus involve exposure misclassification. Strikingly, the observed ORs are similar as seen in the INTERPHONE main analysis. This may suggest that these seemingly protective results are not only due to selection bias but also to reverse causality. Cases with prodromal symptoms may not have engaged themselves in a communication technology that was new at that time.

Cochlear implants convey information between the external part situated behind the ear, and the internal implant via an RF-EMF signal. The implant is likely used in all waking hours and has a field strength around 20-40 mW typically at 5-12 MHz. Spurred by a case report (Kalakoti et al., 2016) Smeds et al. (2018) formed a cohort of all patients (n=2 748) receiving a cochlear implant in Sweden during the years 1989-2014. Cohort members were required to be between 1 and 89 years of age, have no brain tumour or acoustic neuroma prior to implantation or within one year of operation and to reside in Sweden. The resulting cohort of 2 714 patients was followed up for glioma, meningioma and acoustic neuroma in the Swedish Cancer registry in the period 1990 to 2015. Indirect standardization by sex, age and calendar period was used for calculating standardized incidence ratios (SIR) and 95% CI comparing the number of cases observed and expected from Swedish national incidence rates. The SIRs were 3.16 (95% CI: 0.65-9.24) for meningioma based on 3 observed cases and 0.75 (95% CI: 0.02-4.15) for glioma based on one case. For acoustic neuroma, zero cases were observed and 0.09 expected. The authors conclude that the findings do not support an association between cochlear implants and neurological tumours and point to the major limitations of the study being the low number of cases and that the follow-up period may be too short.

This is one of the few prospective cohort studies on cancer and RF-EMF exposure to the brain. Peak RF-EMF exposure from mobile phones is higher than exposure from cochlear implants, but in terms of cumulative absorbed dose, the contribution of implants is expected to be relevant due to longer transmission duration per day. A limitation is the small sample size and accordingly the low number of cases. No confounders were considered.

In an Italian study from Sardinia, the association between environmental exposure RF-EMF and the risk of lymphoma subtypes was investigated in a case-control study of 322 patients diagnosed between 1998 and 2004 and 444 controls (Satta et al., 2018). Self-reported distance of place of residence from fixed radio-television transmitters and mobile phone base stations was inquired by interview. Further, the address was geo-referenced and RF-EMF was modelled for each address within a 500-meter radius from a mobile phone base station. RF-EMF measurements at the door were conducted in a subset of the longest held addresses within a 250-meter radius. Incident cases aged 25-74 years were identified in two participating hospitals (participation rate: 90.4% of eligible cases). Controls were randomly selected from population registries (participation rate: 59.6%). Data was analysed with unconditional logistic regression, adjusted for age, gender and years of education. In the analysis of self-reported data, residence in proximity (<50 m) to a fixed radio-television transmitter was associated with an increased risk for lymphoma and for the major lymphoma subtypes. No association was observed for self-reported and geo-coded distance from mobile phone base stations or for estimated RF-EMF exposure. RF-EMF measurements did not vary by case-control status.

This is a carefully conducted study with the limitation that the observed absence of association is

compromised by the limited sample size. Unfortunately, coordinates of radio or television transmitters were not available and it was thus not possible to check the self-reported distance associations with objective radio-television transmitter data. By comparing self-reported and objective distance from mobile phone base station, it was found that cases tended to underestimate the distance from nearest base station. Thus, it is conceivable that also self-reported distance from radio-television transmitter may suffer from recall bias and that observed associations were biased.

Incidence trends for brain tumours

de Vocht (2019) investigated trends in brain tumour subtypes in England over the years 1985 to 2014 expanding a previous analysis (de Vocht, 2016, de Vocht, 2017) with more updated cancer data and inclusion of additional covariates. He investigated all brain tumour types situated in the temporal lobe as well as glioblastoma multiforme (GBM) in different brain regions. The incidence data for 1985 to 2005, in combination with population level data on covariates for the period 1985 to 2014 (UK annual: all cancer and brain cancer incidence, smoking prevalence, urbanization rate, a measure of UK office of national statistics coding quality, population estimates in broad age categories and total number of medical scans) was used to model predicted (counterfactual) incidence trends for the period 2006 to 2014. Any difference between these calculated trends and the actual observed trends could then potentially be caused by mobile phones. He then included also national mobile phone coverage rates in the model to explore to which extent this factor correlated with observed differences between the two trends. For GBM, the observed incidence exceeded the predicted incidence for frontal lobe (cumulative causal impact (CCI): 36%, 95% Bayesian credible interval (BCI) -8% to 77%), temporal lobe (CCI: 38%, 95% BCI: -7% to 78%) and cerebellum (CCI: 59%, 95% BCI: 0 to 120%). For tumours of the temporal lobe, excess rates were observed for all malignant tumours (CCI: 33%, 95% BCI: 10% to 54%), unspecified glioma (CCI: 175%, 95% BCI: 17% to 373%), GBM (CCI: 38%, 95% BCI: 7 to 78 %) and anaplastic astrocytoma (CCI: 42%, 95% BCI: 8 to 79%). Including mobile phone coverage rates reduced the CCI for GBM of frontal and temporal lobes and for malignant neoplasms and glioma of the temporal lobe suggesting an association. However, the discrepancy between observed and expected cases increased by age, reaching CCI=177% (95% BCI: 100 to 252%) among those aged 85 or older, who were unlikely to be early users of mobile phones. De Vocht concludes that although the trend with increasing incidence of GBM of the temporal lobe appears consistent with the hypothesis of mobile phones being causal, the increase is likely associated with other factors. Improved diagnostics especially in the elderly represents the most plausible candidate. The suggestion of a change in tumour types over time is in accordance with other incidence studies. The study is ecological in nature without any individual phone usage data in nature and heavily dependent on the quality and availability of indicators from which to calculate the counterfactual trends. This is the first study that tried to control for secular trends using a Bayesian framework. Nevertheless, it was impossible to discern if association were caused by the phones or by other factors changing over the same time period such as improved diagnostics or changes in coding praxis over time.

Li et al. (2018c) investigated incidence trends of diffuse glioma among adults (age ≥ 20) in the US over the years 1973-2014 using SEER-9 data, covering around 9.5 % of the US-population and including a total of 49 124 primary glioma patients. The overall age-standardized incidence rate of glioma increased until 1985, after which it exhibited a small decrease. The relative distribution of tumour types changed over time with glioblastoma increasing from 1978 whereas the proportion of non-glioblastoma decreased from 1982. The authors point to changes in classification as well as improved diagnostic imaging as explanatory factors for the changed composition of glioma subtypes. With regard to mobile phones, it is noteworthy that glioma did not increase since 2015 and the birth cohort analysis did not find marked variations among those born after 1930s, including the part of the population likely to include early mobile phone users. This study indicates that analysis of specific histologic subtypes may be affected by changes in coding praxis and should always be interpreted by considering temporal trends in all subtypes belonging to the same tumour type.

Karipidis et al. (2018) investigated incidence trends of brain tumours among adults aged 20-59 in Australia in the period 1982-2013. A total of 16 825 cases (93.7% glioma) were identified and annual

percentage change (APC) in incidence calculated separately for the periods 1982-1992, 1993-2002 and 2003-2013. During the first period, there was an increased incidence of high-grade glioma and glioma with specified locations (APCs between 3.8 and 7.8). The intermediate period (1993-2002) saw increases in glioblastoma, high-grade glioma and glioma of the frontal and temporal lobe (APCs 2.1-3.7). In the third time period (2003-2013), the only increase was for glioma of the frontal lobe (APC 3.0: 95% CI: 1.6-4.5). The incidence of unspecific diagnoses tended to decrease and the overall APCs for all brain tumours and for glioma remained close to zero over the three periods. The authors interpreted the three periods as representing respectively: 1. increased use of CT and MRI, 2. improved MRI, 3. Substantial mobile phone use. They argue correspondingly that the improvement of diagnostic procedure is the most likely explanation for the observed changes until 2002 and that the increased incidence for frontal lobe tumours is unlikely to relate to mobile phones as there was no increase in the more exposed temporal lobe in the period 2003-2013 when mobile use was highest. For the period 2003-2013, the authors also compared the observed APC for glioma with modelled scenarios using assumptions of RRs for mobile phone and glioma ranging from 1.5 to 3.0. The annual mobile phone coverage was assumed to be 90% of the total number subscriptions in Australia for each year and assumed equal across sex and age groups. The resulting APC estimates were consistently higher than the observed APCs, calculating latencies up to 15 years. However, for a 20 year latency even a RR of 3.0 would only result in an APC of 0.6 (25% CI: -0.2-1.5). The authors concluded that their results do not support an association between mobile phone use and brain tumours, but calculations did not exclude the possibility of small risks or latencies exceeding 15 years. The study is ecological in nature and particularly the mobile phone scenarios relied on crude data. Also, the narrow age span and the presentation of results as APCs for three periods to some extent impedes comparison with other incidence studies. The results however corroborate that the major changes in incidence within brain tumours largely relate to changes in classification rather than changes in incidence. An interesting finding is also the reduction of tumours with unknown location in contrast to an increase for all other topographies. This indicates that improved diagnosis leads to improved localisation of tumours. Therefore, increases of incidence trends for specific localisations (e.g. temporal lobes) should always be interpreted relative to temporal changes of tumours at other sites including those without localization information.

4.1.2. Self-reported electromagnetic hypersensitivity (EHS) and symptoms

In a French study (Irigaray et al., 2018), various parameters in the blood of 32 electromagnetic hypersensitive (EHS) individuals were analysed to evaluate oxidative and antioxidative stress response. The mean age of the EHS sample was 51 years (32 to 75 years) and 69% were female. One parameter (nitrotyrosine) was only measured in 14 individuals and for the corresponding analysis, data was pooled with a concomitant sample of EHS self-reporting patients (n=46, mean age=49 years, 71% female). Blood parameters of the EHS sample was compared with normal values obtained in healthy controls by means of a T-test without considering any covariates. Depending of the biomarker considered, 30-50% of EHS had increased levels (thiobarbituric acid-reactive substances, malondialdehyde; oxidized glutathione, nitrotyrosine). In contrast, decreased levels with no measurements above the upper normal limits was observed for glutathione (GSH), oxidized glutathione (GSSG), GSH/GSSG ratio, total glutathione (GluT) and GSH/GluT ratio. Furthermore, red blood cells, mean Cu-Zn superoxide dismutase and glutathione peroxidase activities were increased in ca. 60% and 19% of the patients, respectively, while increased glutathione reductase activity was observed in 6% of the patients. The authors conclude that about 80 % of self-reporting EHS patients have at least one oxidative stress biomarker above the normal range indicating a pathological disorder. No information is given about the blood sampling scheme in EHS individuals. There is also no information about the referent group and whether they are comparable with the EHS sample in terms of age, gender and lifestyle. Control reference values are not referenced. The observed difference of biomarkers may be due to sampling differences, or because the EHS collective represents a symptomatic sample of the population. It is also not clear what the proportion outside normal range would be in a healthy collective and whether the reported proportions for EHS are to be expected for

any other (healthy) population. Most important, values outside the reference range does not imply that the health issues were caused by the exposure to electromagnetic fields. No EMF exposure assessment was conducted in this study.

A prospective cohort study in China addressed the association of long-time mobile phone use with sleep disturbances and mental distress in technical college students (Liu et al., 2019). A total of 4 333 students (response rate: 91.5%) participated at baseline and 3 396 students (78.4%) could be included in a follow-up investigation 8 months later. Data were collected by a set of questionnaires including socio-demographics, lifestyle, various sleep variables, Beck Depression Inventory, Zung Self-Rating Anxiety Scale and duration of mobile phone use per day, which included different aspects of mobile phone use (calling, texting, watching video, etc.). Analyses were adjusted for numerous potential confounding factors and covariates. At baseline, participants were asked “how long do you usually spend on using mobile phone per day?”, which likely includes all kind of activities and not only calling. In total, 23.5% of the sample reported to use a mobile phone for ≥ 4 hours/day. Such behaviour was positively associated with incidence of sleep disturbance and mental distress at follow-up. Conversely, a reduction of the amount of mobile phone use at follow-up compared to baseline was associated with an improvement of these health-related outcomes.

This is a large study that followed a longitudinal design. This study confirms results from previous studies that excessive electronic media use is related to a higher risk for reduced mental health and sleep problems. In their analyses, the researchers did not differentiate between mobile phone activities producing high RF-EMF exposure such as calling and activities resulting in low RF-EMF exposure such as texting. Thus, the study did not aim at investigating effects from mobile phone radiation but rather addressed the effects from mobile phone use per se. Thus, the study is not able to reveal whether RF-EMF exposure contributed to the observed associations.

In a cross-sectional study, the relationship between mobile phone use and mental health was examined among university students in Serbia and Italy from March to May 2016 (Visnjic et al., 2018). A total of 785 students of both genders were included; the participation rate was not reported. Symptoms were asked using the Depression Anxiety Stress Scale (DASS 42). Logistic regression was conducted without considering any covariates. Depressive symptoms increased with increasing number text messages and decreased with duration of calls and duration of internet browsing. Anxiety symptoms also increased with increasing number of text messages and decreased with duration of internet browsing and duration of e-gaming. Stress symptoms were negatively correlated with number of calls and duration of e-gaming and positively correlated with time spent on the phone. Stress symptoms were also elevated in students reporting to have the phone closer than one meter during sleep. As every cross-sectional study, this study is not able to reveal what came first, i.e. whether existing symptoms affected mobile phone use, or the other way round. Lack of consideration of covariates in the statistical analysis is a limitation of this study and it is thus not clear whether the observed associations are due to other factors. If real, the pattern clearly does not indicate a role of RF-EMF exposure but rather other aspects of mobile phone use such as extensive social interactions (texting) or mobile phone related awakenings due to e.g. incoming messages on a mobile phone kept close to the bed.

4.1.3. Other outcomes

Bektas et al. (2018) investigated the association between maternal mobile phone use and various parameters in postnatal cord blood in 149 pregnant women aged 18 to 40 years who did not undergo any medical treatment, had chronic systemic disease or had multiple pregnancy. Pregnant women were grouped into non-users of mobile phones ($n= 37$), low users (2–15 min/d, $n= 39$), moderate users (15–60 min/d, $n=37$) and heavy users (60 min/d, $n=36$). For all assessed blood parameters (aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatine kinase, creatine kinase–myocardial band, c-reactive protein, procalcitonin, troponin T, uric acid and lactate levels, platelet volume), significant differences were observed, mostly in a exposure-response like manner. No covariates were considered in this study. Thus, it remains unclear whether group differences were

due to mobile phone use or because of other factors which may differ between groups such as lifestyle or environmental exposures. No information is given about how the women were recruited and what the participation rate in the study was.

The study by Sudan et al. (2018) addressed the question whether mobile phone use of women during pregnancy could lead to cognitive deficits in their children at age five. The authors based their analysis on cohort data from three countries (Denmark, Spain and Korea) with a total of 3 089 participating mother-child pairs. Both maternal cell phone use and child behavioural problems were assessed through questionnaires filled in by the mothers. In Spain and Korea, mobile phone use was assessed during pregnancy, whereas in Denmark, mothers were asked about their mobile phone use during pregnancy when the children were 7 years of age. Children's cognition was assessed at ages 4–6 years using the Wechsler Preschool and Primary Scale of Intelligence - Revised (Denmark and Korea) or the McCarthy Scales of Children's Abilities (Spain). Linear regression analyses were adjusted for numerous covariates. Models were computed separately for each cohort and then meta-analysed using random effects. Overall, no association between general, verbal, and non-verbal cognition scores and frequency of prenatal mobile phone use was observed. Scores tended to be lower in the highest frequency of use category in the Danish and Spanish cohort but not in the Korean cohort. The study is well conducted and the sample size is relatively large. Prospectively collected mobile phone use data in two of the cohorts is an asset of this analysis. Note that RF-EMF exposure of foetus during pregnancy is very low and it is not yet demonstrated that maternal mobile phone use is correlated with RF-EMF exposure of the foetus at all. Thus, a plausible biological mechanism why there could be an association between maternal mobile phone use during pregnancy and cognitive function is missing. Different result pattern between the Asian and European cohorts may indicate that residual confounding may be relevant.

The Dutch study by Guxens et al. (2019) addressed the association between RF-EMF at the place of residence, mobile and cordless phone use and behavioural problems in a sample of 3 102 children aged 5 years. A validated propagation model was used to predict RF-EMF exposure from a mobile phone base station at the place of residence. Information about indoor sources such as WLAN and cordless mobile phone base station as well as mobile and cordless phone use was obtained by a questionnaire. Parents and teachers filled in a validated questionnaire on various aspects of behavioural problems (Strengths and Difficulties Questionnaire, SDQ). Numerous covariates were considered in the statistical analyses. Mobile and cordless phone use was not associated with behavioural problems. High RF-EMF exposure from mobile phone base stations was associated with more frequent emotional symptoms according to the parental report but not according the teachers' report. Children with a cordless phone base station at home showed less often a prosocial behaviour according to the teachers and had more often maternal-reported peer relationship problems. Children who watched television for at least 90 minutes a day expressed more maternal-reported hyperactivity/inattention behaviour.

The large sample size is an asset of this study and a high number of potential confounders were considered in the analysis. Nevertheless, it cannot be ruled out that the sporadic observed associations occurred by chance or due to other factors, which were not considered in the analysis. The few associations are inconsistent in terms of maternal and teachers' report. No information is given about the extent of mobile phone base station exposure. Children at the age of five years did not use mobile and cordless phones a lot. Accordingly, the absence of associations for mobile and cordless phone use might not be significant for long duration of mobile phone use.

The study by Foerster et al. (2018) investigated the relationship between absorbed RF-EMF dose from wireless communication devices and memory performance in adolescents. The study follows up a report published by Schoeni et al. (2015) with twice the sample size and more recent information on the absorption of RF-EMF in adolescents' brains. Almost 700 adolescents aged 12 to 17 years participated in the study over a period of one year. The participants were recruited from 7th to 9th public school grades in urban and rural areas of Swiss-German speaking Switzerland. Figural and verbal memory performance was measured twice with a one-year follow-up period using standardised computer tests. In addition, with the consent of the parents and the adolescents, the analysis included

objectively collected mobile phone usage data from the Swiss mobile service providers, covering the entire study period. Environmental RF-EMF exposure was individually modelled for the school and residence of the study participants. A subgroup of the adolescents also participated in a personal RF-EMF measurement study. Based on these usage and exposure data, the cumulative RF-EMF dose from mobile phones and other wireless communication devices (expressed in mJ per kilogram body weight per day) was calculated both for the brain and for the whole body (Roser et al., 2015). The study found that cumulative RF-EMF brain dose from mobile phone use over one year was associated with a negative effect on the development of figural memory performance in adolescents, confirming prior results published in 2015. Figural memory is mainly located in the right brain hemisphere, and association with RF-EMF was more pronounced in adolescents using the mobile phone on the right side of the head (80% of study participants). Verbal memory is mainly located in the left brain hemisphere. With regard to usage data from mobile service providers, adolescents using their mobile phone also on the left side of the head tended to show a negative effect on the development of their verbal memory. Other aspects of wireless communication use, such as sending text messages, playing games or browsing the internet cause only marginal RF-EMF dose to the brain and were not associated with the development of memory performance over one year.

The dependence of the results on the laterality and absence of associations in the negative exposure control variables texting, gaming and browsing the internet may suggest that RF-EMF absorbed by the brain is responsible for the observed associations. Most of the cumulative brain dose was from own mobile phone calls, while the contribution of mobile base stations and Wi-Fi was low. A strength of the study is the assessment of cumulative brain dose and the use of objective mobile phone use data recorded by mobile service providers. The effects were relatively small and the mechanism of action is unclear. An influence of other factors thus cannot be completely ruled out. For instance, the study results could have been affected by puberty, which affects both mobile phone use and the participant's behaviour as well as cognitive abilities. The study sample is relatively small.

In a study from Saudi Arabia (Meo et al., 2019) cognitive function of students from two different schools differently exposed to mobile phone base station radiation were compared. RF-EMF was measured with repeated spot measurements using a Narda Safety Test Solution device SRM-3006. In School 1, RF-EMF exposure was 2.7 V/m and in school 2 it was 6.1 V/m. Out of 300 invited students, 217 were included in the study after reviewing their clinical history. Cognitive function was measured with the Cambridge Neuropsychological Test Automated Battery (CANTAB). Data was analysed using a student T-test without considering any covariates. Significant impairment in Motor Screening Task and Spatial Working Memory was observed among the group of students who were exposed to high RF-EMF levels at school.

The study has several limitations. RF-EMF exposure was quite high in both schools and the difference was relatively small compared to exposure variation which can be expected within each school. Further, exposure from other sources (e.g. own mobile phone use) was not considered, as was exposure from home. Thus, it remains unclear whether there is a real exposure difference between students from school 1 and school 2. The students between the two schools may differ in terms of their selection (e.g. difference in socio-demographic factors), impacting their cognitive ability, but no covariates were considered in the analysis. The proportion of students excluded due their medical history was quite high and this may have introduced selection bias.

Vanishree et al. (2018) recruited 30 males and 30 females age 20 to 28 among outpatients from Navodaya Dental College and Hospital, India. Subjects were classified into two groups "high mobile phone users" (more than 5 years of use and more than 10 h/week) and "low mobile phone users" described as using a mobile phone for <5 years and <4-5 h/week. The other specified inclusion criteria stated "healthy individual in the age limit 20-28 years" and "Receiving and making calls was considered". Participants with oral lesions or "deleterious habits" were excluded. Percentage of micronuclei was calculated among 1000 cells in smears from the right and left cheek of each participant. Using unpaired t-tests, there were significant differences in mean micronucleus counts in all comparisons e.g. when comparing high vs low users and when comparing the two cheeks according to which was typically closest to the mobile phone. The authors conclude that mobile phone use can cause genotoxicity. They suggest radiation and heating from radiation or from the pressure of the

phone as possible causes.

The study has several limitations, which may have influenced the results. There is no mentioning of any blinding procedures, there is no table or other information to allow the reader to assess if the groups were similar in terms of socio-demographics or other potentially relevant factors, and the statistical methods did not account for potential confounders. In addition, the description of the study base is unclear; some criteria seem conflicting (low use is lower than the stated inclusion criteria “minimum range of 4 h or above was considered”) and some criteria were only described in very broad terms such as “deleterious habits”.

Bogers et al. (2018) performed a pilot ecological momentary assessment study among seven self-declared electromagnetic hypersensitive individuals. Participants attributed their symptoms beforehand to a clearly defined RF-EMF source and used an exposimeter to collect exposure information during 21 days. In addition, during the same time period, participants reported their symptoms: an electronic diary triggered participants to fill in health related information every six hours in the morning, afternoon and evening. Symptoms that were attributed by the respective individual to RF-EMF exposure were added to the diary. Associations between exposure and symptoms were only evaluated for 5 of the 7 participants; for two participants either the symptom score did not change over time, or there were too many missings in the health-related diary. For four of the seven participants, correlations between the exposures in the six hours before filling in the health questionnaire were observed, of which some were negative and some were positive. The authors concluded that their approach was feasible, but that their results may have been affected by residual confounding. This is because both symptom reporting and RF-EMF exposure may depend on location and activity, but these factors were not taken into account in the analysis. The authors recommended choosing shorter time intervals for future assessments. Overall, the results are difficult to interpret, given the potential for confounding and because evaluated exposure metrics appeared to be chosen relatively arbitrarily. For example, no good explanation is provided as to why the rate of change metric should be a possible cause of symptoms rather than higher average exposures. Note that such an approach may also suffer from placebo effects, if participants’ knowledge about the exposure situation correlates with measured exposure levels.

4.1.4. Conclusions on epidemiological studies

With respect to mobile phone use and brain tumours, various analyses of cancer incidence time trends did not observe patterns supporting the hypothesis of increasing incidence rates following, with some latency, the time period of mobile phone uptake. These new incidence studies demonstrate changes between diagnostic or topographic classification over times. For instance, glioblastoma incidence has been increasing in the US but at the same time other brain tumour diagnoses have decreased. As it is unlikely that RF-EMF exposure from mobile phones is protective for some tumours and presents a risk for others, this rather indicates it is a result of changing coding praxis over time. Similarly, determining the location of tumours in the head has improved over time due to improved imaging techniques, which in turn resulted in seemingly increasing rates of tumours at specified sites of the head, including lateral sites. In contrast, incidence of brain tumours with unknown location has decreased over time. Again, this is an indication of changes in diagnostic and coding praxis and not the consequence of mobile phone related exposure. These new studies demonstrate that possible changes in coding praxis over time needs to be considered in a meaningful manner when interpreting time trends of specific subgroup diagnoses.

A new study on mobile phone use and survival time of glioma patients in Sweden, Denmark and Finland did not find indications that mobile phone user have a shorter survival time, which would indicate a cancer promoting effect of mobile phone RF-EMF exposure. This finding is in contrast to a previous Swedish study by Carlberg and Hardell (2014). The new study, however, indicated that cases with a poor prognosis were less likely to start mobile phone use shortly prior to their diagnosis, probably due to already existing symptoms. This type of bias may also explain decreased odds ratios for regular users seen in the Interphone papers.

New studies on mobile phone use and media use in relation to health-related quality of life, cognitive function and behaviour of children and adolescents often report associations. Some studies point to other exposures related to media use, but not RF-EMF, as a causal factor since the strongest associations were found with e.g. texting, which causes minimal amounts of exposure. These studies show that it is challenging to separate effects from RF-EMF exposure from other aspects of mobile phone use such as being woken up during night, blue light exposure or addictive behaviour. This is especially the case when dealing with outcomes like health-related quality of life, cognitive functions or behaviour. A few attempts in this direction have been done by explicitly calculating the dose absorbed by the body and doing analyses also with so called negative-exposure control variables. Negative exposure control variables refer to activities, which produce little RF-EMF exposure (e.g. texting) but may be a good surrogate for other exposures like being woken up during night, blue light exposure or addictive behaviour. A Swiss study found indications for an RF-EMF effect on memory performance. However, this observation needs to be confirmed in other populations applying a similar RF-EMF dose approach. New studies on other outcomes than discussed were not very strong from a methodological perspective and no firm conclusions can be drawn.

4.2. Human studies

As for previous reporting periods the number of studies addressing RF-EMF effects in human experimental studies is higher than for other exposure types. In the current reporting period four human studies investigating possible effects of radiofrequency electromagnetic field exposure on the autonomic nervous system (Misek et al. 2018, Selmaoui et al. 2018), thermal pain threshold (Vecsei et al. 2018), and symptoms (Eltiti et al. 2018) were published.

4.2.1. Autonomic nervous system

In the reporting period two papers addressed RF-EMF effects on the autonomic nervous system. Selmaoui et al. (2018) focussed on electrodermal activity (EDA) in response to an auditory stimulus as outcome parameter, while Misek et al. (2018) focussed on the heart rate variability (HRV) during an ortho-clinostatic test (i.e. a slow transition from lying to standing and back).

Selmaoui et al. (2018) recorded the electrodermal activity (EDA) - also known as skin conductance (SC) - in response to an auditory stimulus to explore RF-EMF effects on the autonomic nervous system. 28 healthy young volunteers (14 females and 14 males, age range: 19 – 31 years) were exposed to sham and EMF from a GSM mobile phone over two sessions in a randomised, double-blind, counterbalanced study design. The test sessions, that lasted 61 min and included an exposure for 26 min 15 s, were separated by at least one week and took place at the same time of the day. The maximum SAR values averaged for 10 g tissue, 1 g tissue or the peak value were 0.49, 0.70, and 0.93 W/kg, respectively. The acoustic stimulus was a signal tone of 60 dB and 1000 Hz lasting for 0.3 s with an interstimulus interval of 15 s. Artefacts were visually inspected. Besides amplitudes of tonic and phasic activity several variables related to skin conductance responses were calculated. None of the outcome parameters was affected by RF exposure indicating that sympathetic activity, one of the main branches of the autonomic nervous system, is not altered by RF fields from mobile phones.

Misek et al. (2018) investigated effects of an intermittently applied 1788 MHz pulsed RF signal on heart rate variability (HRV). The study included 46 healthy grammar school students (16 males, 30 females, 16.7 to 23.3 years) of which 23 were exposed to sham twice and the other 23 were exposed both to sham and real. The samples did not differ in basic characteristics like age, gender distribution, blood pressure and outcome parameters before the trial. Exposure was delivered by two identical loop antennas, for exposure in the two different positions of the test (standing and lying). The antennas were positioned 30 cm from the right side of the student's face, resulting in a max SAR_{10g} of 0.405

W/kg, which did not lead to an increase in tympanic or skin temperature. All exposures were delivered single blind; however, data evaluation was performed blinded to the exposure condition. The protocol started with a 15 min rest followed by the assessment of basic parameters (e.g. blood pressure and temperature), these measurements were repeated after the end of the exposure session. The experiments, which were performed in the morning (08:00 – 11:00) to avoid circadian variation, consisted of two consecutively performed exposure sessions. The first one always was a sham exposure followed by another sham exposure or a real exposure. In every session recording of outcome parameters started with a 5 min lying condition, followed by 5 min standing and again 5 min lying. Besides subjective perception of exposure seven measured outcome parameters were considered: the spectral power of low and high frequencies in the signal and the ratio between them, the total spectral power, the respiration rate, the RR interval of the ECG and the root mean square of successive differences (rMSSD). None of the subjects was able to distinguish between real and sham exposure. No parameter showed exposure-related differences in the standing position. However, in the lying position two parameters were affected: RF-EMF exposure led to a rise in the power of the high frequency band of HRV and the rMSSD, indicating an increase in parasympathic nerve activity. The argument for RF-EMF related effects would have been stronger if the same results could have been observed between the RF-exposure condition in the second session and the sham condition in session one. Unfortunately, the authors do not report results for such a comparison.

4.2.2. Thermal pain threshold

Several years ago, Vecsei et al. (2013) have shown, that a 30 min Universal Mobile Telecommunication (UMTS) exposure emitted from 3G mobile phones led to an acute, mild pain desensitization shift of thermal pain threshold in healthy young adults in a double-blind, placebo-controlled crossover design. To confirm that RF-EMF exposure affects the thermal pain threshold (TPT), the authors used the same methodology as in the UMTS study, which had previously been validated using the capsaicin-induced hyperalgesia model, to investigate the effect of a 30 min Long Term Evolution (LTE) exposure with RF emitted from a 4G mobile phone (Vecsei et al., 2018). Eighteen healthy adults (12 females and 6 males, age range 19 – 26 years) participated in the double-blind placebo-controlled counterbalanced crossover study. The temperature stimulus was increased by 5 °C/s from 25 °C to a maximum of 55 °C. For the experiment 1750 MHz was used as carrier frequency with 20 MHz bandwidth. The maximum peak SAR as measured in a Specific Anthropometric Mannequin (SAM) phantom was 1.8 W/kg and the distance of the patch antenna from the ear was 7 mm. The real and the sham exposure sessions were scheduled with an interval of at least one week between assessments. For each participant experiments were carried out at the same time of the day. Deviating from the previous results with UMTS exposure no LTE exposure effects on peripheral TPT were observed. No RF exposure effects are in line with other results, which shows that sensitization to pain attributed to mobile phone use usually reflects the attitude towards mobile phone use and thus primarily nocebo or placebo effects.

4.2.3. Symptoms

The nocebo effect was also investigated in a study by Eltiti et al. (2018) who re-analysed data from two previous double-blind provocation experiments performed in individuals with idiopathic environmental illness (IEI) who attributed their symptoms to electromagnetic field exposure, and in control subjects. While the original analysis focussed on effects on symptoms from exposure to a GSM/UMTS base station signal (Eltiti et al., 2007) and a TETRA base station signal (Wallace et al., 2010), respectively, the focus of the re-analysis was on effects of participants' thought as to whether they believed they were exposed or not. As expected IEI-EMF participants reported a significantly lower level of subjective well-being when they believed that the base station was "on" as compared to "off". However, control subjects, who do not believe, that they are affected by RF-exposure, did also report more symptoms when they believed that they were exposed suggesting that control subjects are also susceptible to this nocebo effect, although to a lesser extent.

4.2.4. Conclusion on human studies

None of the four human experimental studies on RF-EMF effects, which were published in the reporting period and which addressed various outcome parameters (electrodermal activity, heart rate variability, thermal pain threshold, and symptoms) did observe exposure effects. These studies thus add evidence to the conclusion that there are no adverse short-term effects of RF-EMF exposure.

4.3. Animal studies

The trend from previous years to study oxidative stress continued, and now included are studies on brain, testes, kidney, liver and eye. Other effects on the brain and effects on behaviour also continue to attract research interest, as do investigations on male fertility.

4.3.1. Brain and behaviour

Karimi et al. (2018) exposed Sprague Dawley rats aged 8-9 weeks in groups of 10 or 11 to 2.45 GHz continuous wave fields for 2 h per day during 40 days at a power density of 0.016 mW/cm² (0.16 W/m²). Exposed animals showed slower learning in a radial maze test ($p < 0.01$) and a higher number of errors ($p < 0.001$). In addition, their performance in a passive avoidance test was decreased ($p < 0.001$). The excitability of pyramidal neurons was decreased ($p < 0.05-0.001$) and long-term plasticity impaired ($p > 0.001$). Histological analysis revealed a decreased number of pyramidal neurons in the exposed animals ($p < 0.01$).

A SAR level was provided, but this was incorrectly calculated using the external E field.

Keleş et al. (2018) exposed 3-week old Sprague Dawley rats ($n=8$ per group) to a 900 MHz field for 1 h per day during 25 days at an whole-body SAR of 0.01 W/kg. At the end of the exposure period, no significant changes were observed in learning, memory or locomotor behaviour as assessed with the passive avoidance, 8-arm radial maze and Y-maze tests. Histopathological examination of the hippocampus showed some damage of pyramidal and granular cell structures in the exposed group, but this was not quantified.

Ahmadi et al. (2018) used 10-12 week old Wistar rats to investigate the effect of EMF exposure on memory. The animals were exposed to a 900 MHz signal from a GSM mobile phone for 42 min per day for 4 weeks. Each exposure consisted of 50 sequences of 35 s at 417 mV/m and 15 s at 17 mV/m measured at the centre of the cage. In a passive avoidance test, memory was shown to be impaired in the exposed group ($p < 0.001$). After administration of naloxone, the impairment of memory performance induced by the EMF exposure was reduced. The authors concluded that memory impairment resulted from activation of the opioidergic system in early memory consolidation as well as deactivation of the nitrenergic system in the retrieval phase of memory.

Gupta et al. (2018) exposed Charles Foster rats ($n=6$ per group) to 900, 1800 and 2450 MHz fields resulting in whole-body SARs of 0.02, 0.03 and 0.06 W/kg, respectively. Exposure was for 1 h per day during 28 days. No effects on exploratory behaviour and anxiety were observed for any frequency at 1, 7, 14 and 21 days of exposure. After 28 days of exposure an impairment of exploratory behaviour and an increase in anxiety were seen with 2450 MHz ($p < 0.05$). In that same group a decrease in acetylcholine and an increase in acetyl cholinesterase in the hippocampus were observed, indicating impairment of cholinergic system, as well as loss of mitochondrial function and integrity, an increase in amyloid beta expression, release of cytochrome-c and activation of apoptotic factors such as caspase-9 and -3.

Ahmed et al. (2018) studied the effect of an 1800 MHz mobile phone-type signal on neurotransmitters in the brain of young and young adult Wistar rats ($n=7$ per group). The animals were either 1 or 3 months old at the start of the exposures, which lasted 1 h per day during 1, 2 or 4 months with an SAR of 0.843 W/kg in the head. A fourth group was left for 1 month after the 4-month exposure to assess

any recovery. After the exposure they assessed the amino acid neurotransmitters glutamic acid, aspartic acid, gamma aminobutyric acid, glycine, taurine, and the amide glutamine in the hippocampus, striatum, and hypothalamus. They observed significant (at $p < 0.05$) changes for several combinations of neurotransmitter, location, exposure period and recovery time, but without a clear and consistent pattern.

In contrast to what the authors state, these changes were not clearly different between the adult and younger animal groups (but this was not tested).

Jeong et al. (2018) exposed middle-aged C57BL/6 mice (14 months old, 12 per group) to 1950 MHz fields for 2 h per day, 5 days per week during 8 months at a whole-body SAR of 5 W/kg. They observed no effect on locomotor activity.

[Effects on oxidative stress in the brain are described in the next section (4.3.2.)]

Fragopoulou et al. (2018) exposed adult C57BL/6 male mice ($n=8$ per group) to a 1800 MHz GSM field for 2 h at an E field level of 4.3-17.5 V/m. The effects on the hippocampal lipidome and transcriptome profiles were assessed 6 h later. The levels of four fatty acids and two fatty acid sums of saturated and monounsaturated fatty acids were significantly altered ($p < 0.05$) in the exposed group. According to the authors this indicates a membrane remodelling response of the tissue phospholipids. Moreover, they found that the expression of 178 genes was significantly different ($p < 0.05$) from that in the sham-exposed group. These genes were involved in critical biological processes, such as cell cycle, DNA replication and repair, cell death, cell signalling, nervous system development and function, immune system response, lipid metabolism, and carcinogenesis.

Hisdisoglu et al. (2018) exposed rats ($n=12$ per group) to 217 Hz modulated 2.1 GHz fields for 2 h per day during 7 days at a brain SAR of 0.27 W/kg. At the end of the exposure, auditory evoked potentials (AEPs) were recorded using implanted electrodes. Furthermore, oxidative stress parameters were evaluated (these are discussed in section 4.3.2). Peak-to-peak amplitudes of AEPs, evoked power, inter-trial phase synchronization and auditory evoked gamma responses were significantly higher in the exposed group, indicating improvement of processing of auditory signals.

4.3.2. Genotoxicity, oxidative stress

In a study also described in the previous section, Jeong et al. (2018) exposed middle-aged C57BL/6 mice (14 months old, 12 per group) to 1950 MHz fields for 2 h per day, 5 days per week during 8 months at a whole-body SAR of 5 W/kg. This treatment did not change the levels of oxidative stress, DNA damage, apoptosis, astrocyte, or microglia markers in the brain.

Hisdisoglu et al. (2018) exposed rats ($n=12$ per group) to 217 Hz modulated 2.1 GHz fields for 2 h per day during 7 days at a brain SAR of 0.27 W/kg. At the end of the exposure, auditory evoked potentials (AEPs) were recorded (discussed in section 4.3.1). Furthermore, the levels of TBARS (thiobarbituric acid reactive substances) and 4-HNE (4-hydroxy-2-nonenal), and expression of GFAP (glial fibrillary acidic protein), iNOS (inducible nitric oxide synthase), and nNOS (neuronal nitric oxide synthase) were evaluated as oxidative stress markers.

Postaci et al. (2018) exposed 30-34 week old male Wistar rats ($n=9$ per group) to a 2600 MHz field for 1 h per day during 30 days. The SAR in the liver, the organ of interest, was 0.011 W/kg. Semi-quantitative analysis showed an increased dilatation of sinusoids and an increased concentration of caspase-3 and TNF- α (tumour necrosis factor) immunopositive cells. No effects of the exposure were observed in the quantification of the indicators of oxidative stress malondialdehyde, superoxide dismutase and catalase in the liver.

Eker et al. (2018) exposed Wistar rats ($n=9$ per group) to an 1800 MHz field at an E field level 6.8 ± 0.1 V/m for 2 h per day during 8 weeks. They assessed gene expression of Hsp27, p38MAPK, EGFR,

and caspase-3 in whole-eye homogenates. Caspase-3 and p38MAPK gene expression was significantly upregulated ($p<0.05$).

A SAR level is provided, but this was incorrectly calculated using the external E field.

Okatan et al. (2018) exposed 34 days-old Sprague Dawley rats ($n=8$ per group) to a 900 MHz field for 1 h per day during 25 days and assessed effects in the kidney. The whole-body SAR was 0.012 W/kg. Various lesions were observed with histopathological analysis in the exposed group (but no quantification was made). No effects of exposure were observed in kidney levels of malondialdehyde, total oxidant status, total antioxidant status and the oxidative stress index, but the apoptotic index in exposed kidneys was increased ($p<0.001$).

Jonwal et al. (2018) exposed or sham-exposed 6-8 week-old male Swiss albino mice to 2.45 GHz for 2 h per day during 30 days at an whole-body SAR of 0.09 W/kg. ROS (reactive oxygen species), MDA (malondialdehyde), GPx (glutathione peroxidase), SOD (superoxide dismutase) and CAT (catalase) were assessed in the testes to measure oxidative stress. Micronuclei in erythrocytes were measured as indicative for DNA damage. Exposure resulted in increased oxidative stress in the testes (increased ROS, MDA and CAT, decreased GPx and SOD), and an increase in micronuclei in erythrocytes. Effects on the testes function and histology are described in section 4.3.4.

Shahin et al. (2018) exposed 12 week-old male Swiss mice to continuous wave 2.45 GHz for 2 h per day during 15, 30 or 60 days at an whole-body SAR of 0.0146 W/kg. They observed significant increases in reactive oxygen species, nitric oxide and malondialdehyde, and a decrease in antioxidant enzymes ($p<0.05$), which became greater with increasing exposure duration. Furthermore the expression of p53, Bax and active-caspase-3 in the testes was found to be significantly up-regulated while the expression of Bcl-xL, Bcl-2, pro-caspase-3 and PARP-1 were significantly down-regulated after exposure in a duration-dependent manner. These observations indicate increased oxidative stress. Effects on the testes function and histology are described in section 4.3.4.

4.3.3. Physiology

Comelekoglu et al. (2018) investigated effects of RF EMF exposure on rat sciatic nerves (which are located in the thigh). They used adult male Wistar rats, which were exposed in groups of seven to 1800 MHz mobile phone-type signals for 1 h per day during 4 weeks. The SAR_{10g} in the sciatic nerve was <0.0008 W/kg. After the exposures, a significant ($p<0.05$) reduction in amplitude and a prolongation in latency of action potentials in the myelinated nerve fibres were observed, as well as an increase in the malondialdehyde level, catalase activity and degeneration. The authors state that these changes are consistent with neuropathy, and they were partially reduced with administration of paricalcitol, a drug that has shown some promise regarding redox homeostasis in preclinical studies.

4.3.4. Fertility

In a study described in section 4.3.2, Jonwal et al. (2018) exposed or sham-exposed 6-8 week-old male Swiss albino mice to 2.45 GHz for 2 h per day during 30 days at an whole-body SAR of 0.09 W/kg. Serum testosterone levels were decreased after exposure and histopathological analysis of the testes showed increased abnormalities in the exposed animals.

Bilgici et al. (2018) investigated the effect of 2.45 GHz exposures on the testes of 5-6 months-old Wistar rats. They exposed the animals ($n=11$ per group) for 1 h per day during 30 days at a whole-body average SAR of 0.023 W/kg. Levels of three interleukins (IL-6, -10 and -32) in the testes were not altered by exposure, but histopathological evaluation revealed a significantly increased necrosis and decreased spermatogenesis ($p<0.05$). Serum levels of IL-6 and C-reactive protein were increased in the exposed group ($p<0.05$), while those of IL-10 and IL-32 were not changed.

The animals were unevenly exposed, however, from an antenna located in front of the head; the SAR for the testes is not given, but can be read from figure 3 to be around 0.005 W/kg.

In a study described in section 4.3.2, Shahin et al. (2018) exposed 12 week-old male Swiss mice to continuous wave 2.45 GHz for 2 h per day during 15, 30 or 60 days at an whole-body SAR of 0.0146 W/kg. The exposure led to altered testicular architecture, decreased seminiferous tubule diameter, sperm count, sperm viability and serum testosterone level ($p < 0.01-0.001$).

4.3.5. Conclusions

The studies on the effects of RF EMF on brain and behaviour showed inconsistent results. Several studies showed impairment of memory, while others, with virtually similar treatments, did not. For example, a study with whole-body SARs of 0.02-0.06 W/kg showed decreased exploratory activity, while in another study with exposure to a whole-body SAR of 5 W/kg no effect on locomotor activity was found. In the same and in other studies also no effects on oxidative stress in the brain were observed. Increased oxidative stress, however, was observed in the eye (exposure ~ 7 V/m), testes (whole-body SAR 0.015 and 0.09 W/kg) and sciatic nerve (local SAR < 0.0008 W/kg), but not in kidney (whole-body SAR 0.012 W/kg). In testes, whole-body SARs of 0.015-0.09 W/kg also resulted in decreased sperm counts and sperm viability and decreased serum testosterone.

These results are in line with the results of animal studies discussed in the previous Council reports. There is a need for systematic reviews of these studies, in particular on the topics of oxidative stress and male fertility, before any conclusions concerning the possible implications for human health can be drawn.

Table 4.3.1. Animal studies on exposure to radiofrequency fields

Endpoint	Reference	Exposure	Duration	Effect
Brain and behaviour	Karimi et al. (2018)	2.54 GHz, 0.16 W/m ²	2 h/d, 40 d	Decreased learning, decreased neuron excitability
	Keleş et al. (2018)	900 MHz, WBA SAR 0.01 W/kg	1 h/d, 25 d	No effect learning
	Ahmadi et al. (2018)	900 MHz, 417 and 17 mV/m	42 min/d, 4 weeks	Impairment of memory
	Gupta et al. (2018)	900, 1800, 2450 MHz, WBA SAR 0.02, 0.03, 0.06 W/kg	1 h/d, 28 d	Impaired exploration, increased anxiety, impairment of cholinergic system, activation apoptosis after 28 d 2450 MHz exposure
	Ahmed et al. (2018)	1800 MHz, head SAR 0.843 W/kg	1 h/d, 1, 2, 4 months	Changes for several combinations of neurotransmitter, location, exposure period and recovery time, no clear and consistent pattern
	Jeong et al. (2018)	1950 MHz, WBA SAR 5 W/kg	2 h/d, 5d/wk, 8 months	No effect on locomotor activity

	Fragopoulou et al. (2018)	1800 MHz, 4.3-17.5 V/m	2 h	Alteration fatty acids, changes expression 178 genes in hippocampus
Genotoxicity, oxidative stress	Jeong et al. (2018)	1950 MHz, WBA SAR 5 W/kg	2 h/d, 5d/week, 8 months	No change in oxidative stress, DNA damage, apoptosis, astrocytes, microglia markers in brain
	Hidisoglu et al. (2018)	2.1 GHz, 217 Hz modulation, brain SAR 0.27 W/kg	2 h/d, 7 d	Changes in auditory evoked potentials, some effects indicative of increased oxidative stress in brain
	Postaci et al. (2018)	2600 MHz, liver SAR 0.01 W/kg	1 h/d, 30 d	No effect oxidative stress in brain
	Eker et al. (2018)	1800 MHz, 6.8 ± 0.1 V/m	2 h/d, 8 weeks	Upregulation Caspase-3 and p38MAPK gene expression, not Hsp27, EGFR, in eye
	Okatan et al. (2018)	900 MHz, WBA SAR 0.012 W/kg	1 h/d, 25 d	No effect oxidative stress, apoptosis in kidney
	Jonwal et al. (2018)	2.45 GHz, WBA SAR 0.09 W/kg	2 h/d, 30 d	Increased oxidative stress in testes, increased micronuclei in erythrocytes
	Shahin et al. (2018)	2.45 GHz, WBA SAR 0.0146 W/kg	2 h/d, 15, 30, 60 d	Increased oxidative stress in testes
Physiology	Comelekoglu et al. (2018)	1800 MHz, sciatic nerve SAR _{10g} <0.0008 W/kg	1 h/d, 4 weeks	Reduction sciatic nerve function, increased oxidative stress
Fertility	Jonwal et al. (2018)	2.45 GHz, WBA SAR 0.09 W/kg	2 h/d, 30 d	Decreased serum testosterone, increased histological abnormalities in testes
	Bilgici et al. (2018)	2.45 GHz, WBA SAR 0.023 W/kg	1 h/d, 30 d	Interleukin levels in testes unchanged, but IL-6 and C-reactive protein in serum increased; increased necrosis in testes and decreased spermatogenesis
	Shahin et al. (2018)	2.45 GHz, WBA SAR 0.0146 W/kg	2 h/d, 15, 30, 60 d	Histological changes in testes, decreased sperm count, sperm viability, serum testosterone

4.4. Cell studies

4.4.1. Adaptive response

The phenomenon of RF-induced adaptive response (AR) has been further investigated in a paper by Falone et al. (2018). The authors exposed/sham-exposed human SH-SY5Y neuroblastoma cells to a 1950 MHz, UMTS signal, at SAR values of 0.3 or 1.25 W/Kg for 20 h to evaluate the induction of DNA migration (comet assay) induced by RF exposure alone and given before a treatment with menadione (MD), a chemical agent inducing DNA damage via reactive oxygen species (ROS) formation. MD has a pro-oxidant action and the authors reported that MD treatment induced an increase in mRNA levels of the antioxidant enzymes superoxide dismutase (SOD2; $p < 0.05$) and a decrease in the expression of glutathione peroxidase (GPX1; $p < 0.05$). In addition, MD treatment also induced DNA damage and a reduction of oxoguanine DNA glycosylase (OGG1) expression, a DNA-repair enzyme.

The results of at least four independent experiments carried out blinded indicated no effects of RF exposure alone, while pre-exposure to RF at both SAR values investigated induced a significant reduction of MD-induced DNA damage. These results confirmed and extended the capability of RF pre-exposure to induce AR in a neuronal cell model challenged with an oxidative stress-inducer. RF pre-exposure was able to revert the MD-induced effects on gene expression of SOD2, GPX1 and OGG1. Thus RF pre-treatment rendered SH-SY5Y cells less susceptible to oxidative stress, likely due to higher ROS scavenging capacities.

4.4.2. Genotoxicity

Primary rat astrocytes, microglia and cortical neurons were employed by Su et al. (2018a) to investigate the effect of 1800 MHz RF, GSM, 4 W/kg SAR, on DNA damage and cellular functions. Cell cultures were intermittently exposed/sham-exposed (15 min on/10 min off cycles) at different culture times on the basis of the cell types and endpoint investigated. In particular, astrocytes were pre-cultured for 24 h and exposed or sham-exposed for 1, 6 or 24 h to evaluate γ -H2AX foci formation and cytokine detection. The same exposure protocol was applied to microglial cells for the evaluation of γ -H2AX foci formation, cytokine detection (IL-1 β , IL-6 and TNF- α) and phagocytic activity. Cortical neurons were pre-cultured for 7 days and exposed or sham-exposed for 24 h to evaluate γ -H2AX foci. In addition, the latter cell type was also subjected to a pre-culturing of 1 day, followed by 14 day of exposure or sham-exposure, delivered 1 h/day to evaluate axon morphology at day 3, dendrites morphology at day 7 and synapses density at day 14 of exposure. In all cases, three independent experiments were carried out blinded. The results obtained indicated absence of foci formation and of variation in cytokines secretion and of morphological alterations of dendrites or synapses of cortical neurons. However, the exposure significantly reduced the phagocytic activity of microglia ($p < 0.05$) and inhibited the axon length and branch number of cortical neurons ($p < 0.05$).

4.4.3. Autophagy

Autophagy is a catabolic process that facilitates nutrient recycling via degradation of unnecessary or damaged organelles and proteins mediated by lysosomes. It allows degradation and recycling of cellular components and contributes directly to cell metabolism and energy regulation, and is essential for the maintenance of cellular homeostasis.

Li et al. (2018d) evaluated the capability of RF exposure to induce DNA damage, ROS formation and autophagy in mouse spermatocyte-derived cells (GC-2). Cell cultures were intermittently (5 min on/10

min off cycles) exposed/sham exposed to 1800 MHz GSM for 24 h at 1, 2 or 4 W/kg SAR. Each experiment was repeated three times by applying a double blind procedure. The alkaline comet assay showed that RF exposure at 4 W/kg induced DNA damage ($p < 0.01$ compared to sham controls), but such a damage was not detected by the neutral comet assay or the expression level of γ -H2AX foci. Cell cultures exposed at 4 W/kg also resulted in a significant reduction of cell viability compared with the sham-exposed group ($p < 0.01$). In addition, a statistically significant increase in ROS formation was detected ($p < 0.01$), but such an increase was reduced by pre-treatments with melatonin, a well-known antioxidant, indicating that the RF-induced DNA damage is mediated by ROS formation. To investigate the effect of RF exposure on the induction of autophagy, cell cultures were examined by fluorescent microscopy, transmission electron microscopy and by measuring the expression of several protein hallmark of autophagy (western blot analysis). The results showed that RF given at 4 W/kg induced autophagy ($p < 0.01$) and it was mediated via the AMPK/mTOR signalling pathway. By inhibiting such a pathway, autophagy was decreased and DNA damage was increased ($p < 0.01$). Overall, the results indicated that the autophagy, induced by RF-EMFs via the AMPK/mTOR signalling pathway, could prevent the ROS-induced DNA damage in in mouse spermatocyte-derived cells.

4.4.4. Other cellular endpoints

Zhou and co-workers (Zhou et al., 2019) investigated the effect of 2.856 MHz exposure on differentiated rat pheochromocytoma (PC12) cells. Cell cultures were exposed/sham-exposed for 8 h/day for two days at a SAR of 4 W/kg. The results of four independent experiments did not show alterations in ROS formation, induction of apoptosis and intracellular calcium concentration.

4.4.5. Summary and conclusions for cell studies

The new *in vitro* studies confirm the previous Council conclusions: several endpoints have been investigated and in most cases no effect of the exposure was detected. Nevertheless, in some investigations, where high SAR values were considered, effects on some cellular parameters have been reported. As for the past years, several studies have been recognized but not considered due to the scanty quality of the experimental set-up.

Table 4.4.1. Cell studies on exposure to radiofrequency fields

Cell type	Endpoint	Exposure conditions	Effect	References
Human neuroblastoma (SH-SY5Y) cells	DNA damage (comet assay), gene expression	1950 MHz, 0.3 or 1.25 W/kg 20 h	No effects of RF alone. Co-exposure with MD: Decreased MD-induced DNA migration; reduction of MD-induced SOD increase, and GPX1 and OGG1 decrease.	Falone et al (2018)
		Co-exposures with MD		
Primary rat astrocytes, microglia and cortical neurons	DNA damage (foci formation), cytokine secretion, morphological alterations	1800 MHz, GSM, 4 W/kg 1, 6, 24 h (5'on/10'off cycles) 1h/day for 14 days	No effects except for a reduction in phagocytic activity of microglia and inhibition of axon length and branch number of cortical neurons.	Su et al. (2018a)
Mouse spermatocyte-derived (GC-2) cells	DNA damage (neutral and alkaline comet assay, foci formation), ROS formation and autophagy	1800 MHz, GSM, 1, 2, 4 W/kg 24 h (5'on/10'off cycles)	1 and 2 W/kg: no effects. 4 W/kg: DNA damage, detected only with the alkaline comet assay; reduction of cell viability; increase in ROS formation, reverted by treatments with melatonin; induction of autophagy.	Li et al. (2018d)

Rat pheochromocytoma (PC12) cells	ROS formation, apoptosis, intracellular calcium concentration.	2.856 MHz, 4 W/kg 8 h/day for 2 days	No effects.	Zhou et al. (2019)
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Abbreviations: GPX: Glutathione peroxidase MD: menadione; OGG1: oxoguanine DNA glycosylase; ROS: reactive oxygen species; SOD: Superoxide dismutase.

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Appendix: Studies excluded from analysis

Articles were identified in relevant scientific literature data bases such as PubMed as well as in the specialized database EMF Portal. Reference lists of articles were screened for relevant papers. Several studies had to be excluded from further analysis as they did not fulfil quality criteria. In this Appendix, the excluded studies⁷ are listed and the reasons for exclusion are indicated. The list is divided into epidemiological studies, human studies, animal studies and cell studies.

Epidemiological studies

In a first step, all articles that were not relevant for this report were discarded, i.e.

- A) Papers that did not study non-ionizing electromagnetic fields (i.e. static, extremely low frequency, intermediate frequency or radiofrequency EMF), or
- B) did not study any health outcome (including letters, commentaries etc.), or
- C) did not in any way study the association between radiofrequency fields and a health outcome (e.g. use of text messages for self-management of diabetes).
- D) Studies on using EMF as therapeutic interventions (e.g. diathermy),
- E) Case-reports were also excluded.
- F) Further, studies that did not include humans were excluded, as well as studies of humans with an experimental design (these studies are included under “human studies”).
- G) Not a peer-reviewed publication, or published in another language than English,
- H) Studies published outside of the time frame of this report (online publication date).

Further, the following exclusion criteria were applied after screening the abstracts:

- I) Study base not identified (e.g. self-selection of subjects in cross-sectional or case-control studies, the population intended for inclusion not described)
- J) No comparison group or no exposure considered (either no unexposed group or lacking denominator for prevalence/incidence calculation in descriptive or incidence study), with the exception of incidence trend studies from registries applying a systematic data collection.
- K) Narrative reviews
- L) Duplicate reports, unless new additional analyses are presented (including the first original publication, and information from duplicate reports if new additional results were presented)
- M) Addressing exclusively exposure assessment methods which have been proven to be invalid such as self-estimated distance to mobile phone base stations.

Reference	Reason for exclusion
Martens et al. (2018)	A)
Goedhart et al. (2018)	B)
Gourzoulidis et al. (2018)	B)
Brzozek et al. (2018)	B)
Choi et al. (2018a)	B)
Choi et al. (2018b)	B)
Gruber et al. (2018)	B)
Rathebe et al. (2018)	B)
Redmayne (2018)	B)
Reedijk et al. (2018)	B)

⁷ The articles are primarily identified through searches in relevant scientific literature data bases. However, the searches will never give a complete list of published articles. Neither will the list of articles that do not fulfil quality criteria be complete.

Bhatt et al. (2018)	B)
Birks et al. (2018)	B)
Eeftens et al. (2018)	B)
Gallastegi et al. (2018)	B)
Korpinen and Paakkonen (2018)	B)
Kurnaz et al. (2018)	B)
Vuokko et al. (2018)	B)
Zelege et al. (2018)	B)
Lienert P et al. (2018)	B)
Mahmoodi et al. (2018)	B)
Mortazavi et al. (2018a)	B)
Hardell et al. (2018)	B)
Turner et al. (2019)	B)
Mortazavi (2018)	B)
Hosseini et al. (2018)	B)
Ibrahim et al. (2018)	B)
Jung et al. (2018)	B)
Claeson et al. (2018)	B)
Bonato et al. (2018)	B)
Sagar et al. (2018)	B)
Verrender et al. (2018)	F)
Eltiti et al. (2018)	F)
Bachmann et al. (2018)	F)
Deniz et al. (2017a)	H)
Akdag et al. (2018)	I)
Keykhosravi et al. (2018)	K)

Human studies

Radiofrequency (RF) fields

Reference	Reason for exclusion
Henz et al. (2018)	Out of the scope of this report to review studies on any technical devices

Animal studies

Static fields (SF) and extremely low frequency (ELF) fields

Reference	Reason for exclusion
Baltzley and Nability (2018)	GMF, honeybee magnetoreception, no original data (re-analysis of a 1997 paper Kirschvink et al., 1997)
Bobkova et al. (2018)	No sham exposure & group sizes/nos. of mice were not given. Treatment-related (AD).
Bukia et al. (2018)	Treatment-related (epilepsy), insufficient description of exposure.
Chen et al. (2018)	Treatment related (cartilage repair by PMF in a rat model). No sham exposure.
D'Amico et al. (2018)	Scientific review on costs and benefits for bird populations coexisting with powerlines.
Dong et al. (2018)	No in-vivo study, misleading title. Exposure of hippocampal slices

	(collected from rats) to ELF-MF (15-100 Hz, 0.5 -2 mT). Novel patch-clamp set-up.
Kazemi et al. (2018)	No (sham) control in a “descriptive” study using in total 4 rhesus monkeys. Improper description of methods (e.g., daytime of memory tests & of melatonin sampling), and of results (mix-ups of increase and decline).
Kucuk et al. (2018)	Inadequate description of exposure and histotechnology, wire mesh cage housing, no sham exposure.
Li et al. (2018)	Treatment-related (PEMF & bone formation), no exact sham-exposure of hindlimb-suspended rats.
Malewski et al. (2018)	Magnetoreception of three rodent species (Ansell’s & naked mole-rats, C57BL/6J mice) using the MOA (magnetic object assay). No sham-exposure.
Meng et al. (2018)	Technical description of the development of a shield stimulator system for TMS for rodents. No bio-experiment(s).
Mouritsen (2018)	Review on mechanisms in long-distance navigation and magnetoreception in migratory animals.
Novoselova et al. (2019)	Treatment-related (tumor therapy), cross references of text to tables repeatedly incorrect.
Ocal et al. (2018)	Treatment-related (diabetes and vascular complications).
Pal et al. (2018)	Treatment related (spinal cord injury). Iron oxide nanoparticles (IONPs) + ELF-MF. Measurement of MF ?
Rivadulla et al. (2018)	Treatment related (epilepsy, pilocarpine rat model, sensory stimulation). 0.5 T SMF ‘treatment’.
Schweser et al. (2018)	Book chapter describing in vivo and ex vivo protocols for preclinical Susceptibility Weighted Imaging (SWI, MRI) in rodents at 9.4 T.
Sofiabadi et al. (2018)	No description of ELF-MF, no sham exposure. Finally, one exposure group with 3 combined stressors (immobilization, social stress, ELF-MF) does not allow any discrimination.
Sowa et al. (2018)	Proceedings of IEEE Baltic URSI Symposium 2018. Peer review?
Tuhanioglu et al. (2018)	No sham-exposure, diagnostics-/treatment-related.
Vidal-Gadea et al. (2018)	Response comment on “Magnetosensitive neurons mediate geomagnetic orientation in <i>C. elegans</i> ”. No health risk relevant data; no new data. Compare 11 th SSM report 2016 (SSM, 2016).
Wang et al. (2018)	Treatment-related: LF-PPMF, hippocampal neural oscillation, anti-depressive-like effects in rats. Proceedings of EMBC 2018.
Weijers et al. (2018)	GMF, magnetic orientation of cattle. <i>Remark</i> : No confirmation of (south-north) alignment hypothesis.
Yang et al. (2018b)	Exposure description insufficient. Partial description of results of paternal EMP exposure: Female offspring used for other studies and reported elsewhere.
Yang et al. (2018a)	Treatment-related (spaceflight induced bone loss)
Yu et al. (2018)	Technique / strategy how to reconfirm paramagnetic nanoparticles into ribbon-like swarms using oscillating MF).
Yuan et al. (2018)	Treatment related: ELF-MF + cisplatin -treatment sc nephroblastoma (cell line G401) in nude mice
Zhang et al. (2018b)	Treatment-related (bone microstructure and density, growth and development of hindlimbs in mice).

Radiofrequency (RF) fields

Reference	Reason for exclusion
Alimohammadi et al. (2018)	No sham-exposed group, insufficient information on exposure level
Altun et al. (2017)	No sham-exposed group

Bantysh et al. (2018)	No sham-exposed group, exposure level not provided
Deniz et al. (2017b)	No sham-exposed group
Gautam et al. (2019)	No sham-exposed group
Kamali et al. (2018)	Exposure level not provided
Kivrak et al. (2017)	No sham-exposed group
Kocaman et al. (2017)	No sham-exposed group
Masoumi et al. (2018)	Exposure level not provided
Narayanan et al. (2018)	Exposure level not provided
Nasser et al. (2018)	Exposure level not provided
Oh et al. (2018)	Exposure level not clear (2 distances but 1 SAR level)
Pertsov et al. (2018)	No sham-exposed group, no exposure level and frequency
Shojaeifard et al. (2018)	No exposure level and frequency

Cell studies

Static fields (SF)

Reference	Reason for exclusion
Kim et al. (2018)	No sham-control
Ivanova et al. (2018)	No sham-control
Mueller et al. (2018)	No sham-control
Zhang et al. (2018a)	No sham-control
Zheng et al. (2018)	No sham-control
Amiri et al. (2018)	No sham-control

Extremely low frequency (ELF) fields

Reference	Reason for exclusion
Amiri et al. (2018)	No sham-control
Poh et al. (2018)	No sham-control
Song et al. (2018)	No sham-control

Radiofrequency fields (RF)

Reference	Reason for exclusion
Akdag et al. (2018)	No sham-control
Alahmad et al. (2018)	No dosimetry. Cell phone used to expose cell cultures
Houston et al. (2018)	No sham-control
Lamkowski et al. (2018)	No sham-control
Lopez-Furelos et al. (2018)	No sham-control
Mortazavi et al. (2018b)	No sham-control
Shahbazi-Gahrouei et al. (2018)	No dosimetry carried out

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The Swedish Radiation Safety Authority has a comprehensive responsibility to ensure that society is safe from the effects of radiation. The Authority works to achieve radiation safety in a number of areas: nuclear power, medical care as well as commercial products and services. The Authority also works to achieve protection from natural radiation and to increase the level of radiation safety internationally.

The Swedish Radiation Safety Authority works proactively and preventively to protect people and the environment from the harmful effects of radiation, now and in the future. The Authority issues regulations and supervises compliance, while also supporting research, providing training and information, and issuing advice. Often, activities involving radiation require licences issued by the Authority. The Swedish Radiation Safety Authority maintains emergency preparedness around the clock with the aim of limiting the aftermath of radiation accidents and the unintentional spreading of radioactive substances. The Authority participates in international co-operation in order to promote radiation safety and finances projects aiming to raise the level of radiation safety in certain Eastern European countries.

The Authority reports to the Ministry of the Environment and has around 300 employees with competencies in the fields of engineering, natural and behavioural sciences, law, economics and communications. We have received quality, environmental and working environment certification.

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